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VOL. 17, SEC. A. OCTOBER, 1939 NUMBER 10

DISPERSION AND SELECTIVE ABSORPTION IN THE PROPAGATION OF ULTRASOUND IN FLUIDS CONTAINED IN TUBES[1]

By George S. Field[2]

Abstract

A consideration of the transmission of longitudinal sound waves down a fluid-filled tube indicates that anomalous dispersion of the wave may or may not occur at the frequency of radial resonance of the liquid column. In this paper it is shown that the criterion is the rigidity of the tube wall, as radial vibrations will ordinarily appear under the influence of a longitudinal sound wave only when the wall is able to respond appreciably to the alternating pressure occurring in the fluid.

Introduction

A few years ago a series of papers (1, 2, 3) was published dealing with the above subject, with particular reference to a number of experiments that had been carried out with various liquids, in containing tubes with walls both thick and thin in comparison with the radius of the tube. For thin-walled tubes a discontinuity in the curve of phase velocity plotted against frequency was always encountered, in the neighbourhood of the resonant radial frequency of the liquid column. For thick-walled tubes, however, there was no discontinuity, the phase velocity remaining substantially constant with frequency. At the time no explanation for the difference in behaviour of the two types of tubes was advanced. Recently, however, in a comparison of the above results with some data accumulated in connection with other work on air in a glass tube, the real importance of the containing tube has occurred to the author, and is set forth in what follows.

Experimental

In Fig. 1 are reprinted some curves and plotted data from one of the papers (1) in the series mentioned above. It will be observed that the discontinuity in phase velocity which occurs for thin-walled tubes (points plotted as circles) does not exist for the phase velocity of sound in thick-walled tubes (points plotted as crosses).

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[1] *Manuscript received June 20, 1939.*
Contribution from the Division of Physics and Electrical Engineering, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 841.

[2] *Physicist, National Research Laboratories, Ottawa.*

In Fig. 2 are plotted experimental and calculated data for sound in air in a glass tube. In this case the sound was produced at one end of the tube by a high-frequency loud-speaker. Ammonium chloride fume was introduced into the tube and allowed to flocculate. It then collected at the nodal points and made phase velocity measurements possible. Unfortunately, at the time the sound intensity was not sufficiently great to produce useful flocculation at frequencies higher than about 7300 cycles per sec., so that phase velocity measurements were not made after that frequency had been reached.

The vertical broken line in Fig. 2 has been drawn at the frequency corresponding to that of radial resonance of the column of air in the tube. The curve of calculated phase velocities for higher frequencies becomes asymptotic to this line as shown. The curve of phase velocities for frequencies from zero to 24,700 cycles is almost a straight line for most of its length. It drops to the frequency axis at 24,700 cycles, which is the frequency of radial resonance in the wall of the containing tube.

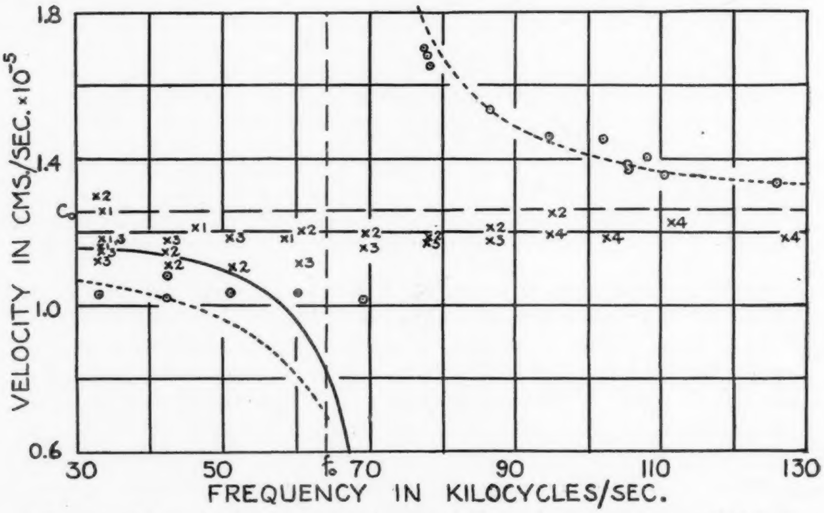

FIG. 1. *Curves showing influence of wall thickness on phase velocity. Naphtha in glass tubes of internal diameter 1.9 cm.: wall thickness, 1.2 mm.; points plotted as circles; wall thickness, 3.3 mm.; points plotted as crosses. The readings were taken on different days, as indicated by the numerals alongside the crosses. The dotted lines represent calculated phase velocities for the thin-walled tube. The solid line is for the thick-walled tube for frequencies below the resonant radial frequency* (f_0) *in the column of liquid.* c_0 *is the velocity of sound in an unconfined volume of naphtha.*

Discussion

At low frequencies the phase velocity of the longitudinal wave in a tube of liquid or gas depends to a very great extent on the rigidity or stiffness of the tube wall. When the acoustic resistance (ρc) of the wall material is not too great compared with the acoustic resistance of the fluid in the tube, or when the wall is very thin, the wall will vibrate with a fairly high amplitude as the result of the alternating pressure in the fluid within. There is a correspondingly great radial vibration of the fluid, and a diminution of the phase velocity of the longitudinal wave progressing down the tube. The theoretical phase velocity drops more and more as the frequency of radial vibration of the tube wall is approached, and eventually goes to zero as this frequency is finally reached. When the tube wall is very rigid, either through its material having comparatively high acoustic resistance or because it is quite thick, its radial motion is very slight; there is only a feeble radial vibration in the fluid column, and the phase velocity of the longitudinal wave is very little different from that of the ordinary plane wave. In fact, in this case the quantity α [see Ref. (2), page 140] is practically equal to zero, and the vibration described by the velocity potential,

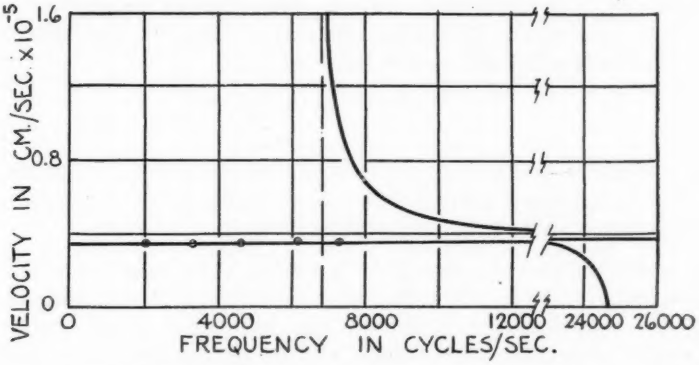

FIG. 2. *Curves showing calculated phase velocities of sound in air in a glass tube of internal diameter 6.2 cm. and wall thickness 1.7 mm. The circles are experimental points.*

$$\phi = AI_0(\alpha r)e^{i\omega(t - z/c_1)}*$$

degenerates to a plane wave, since $I_0(0) = 1$. The longitudinal vibration in the fluid has then a wave velocity independent of frequency.

In Fig. 1 is plotted as a solid line the calculated phase velocity at low frequencies for naphtha in the thick-walled glass tube mentioned under the figure. At 30 kilocycles the calculated velocity of 1.16×10^5 cm. per sec. is slightly lower than the plane wave value of 1.26×10^5 cm. per sec. It will be observed that instead of dropping as the frequency increased, the experimental values remained approximately the same. The theoretical curve, however, is based on equations that neglect the internal friction of the tube wall, and which lead to a very high amplitude of motion at the resonance frequency of the wall. The writer's previous experiments [see Ref. (3) Figs. 1 and 2, and Fig. 1, this paper] have shown that even when a diminution in phase velocity at low frequencies does occur and there is apparently a fairly strong radial vibration in the liquid [see also Ref. (3) Fig. 5 *(a)*], there is no very great drop in phase velocity near the resonant radial frequency of the wall. This indicates that the internal friction in the wall is sufficient to inhibit any large increase in radial amplitude of the wall vibration at or near this resonance frequency. Therefore, for the case of the thick-walled tube, it is not surprising that the phase velocity did not change with frequency. In fact, the wave probably remained essentially plane.

---

* *Ref. (2), Equation 37.*

There is still to be considered, however, the reason for the apparently complete absence of the type of vibration expected to occur just above the absorption band and given by the velocity potential,

$$\phi = AJ_0(kr)e^{i\omega(t-z/c_1)}*.$$

That this vibration was absent for the tube with thick walls is assumed by the lack of high phase velocities at frequencies above that of the calculated radial resonant frequency for the column of liquid.

Here again, the lack of a strong radial motion of the tube wall is probably the explanation. For a tube wall that is relatively thin, it is impossible to propagate a longitudinal wave without the wall "giving" and leading to a radial motion of the particles of fluid. This means that the wave is no longer plane and is given by one or the other of the velocity potentials quoted in this paper, the one which is favoured depending on whether the frequency is above or below the resonant radial frequency of the liquid column. When the fluid is contained in a tube with a thick wall or one having a high acoustic resistance compared with that of the fluid, however, the radial motion of the wall is very slight, and a plane wave is the most easily produced. There is then no variation in phase velocity with frequency for the longitudinal wave in the tube.

The wave given by the velocity potential,

$$\phi = AJ_0(kr)e^{i\omega(t-z/c_1)},$$

may, of course, still be set up in the tube, as such a wave does not definitely require a motion of the tube wall. In other words, this type of wave can exist either with or without a radial motion of the wall, although a plane wave requires that the wall must not move. With a rigid wall, however, this vibration requires to be excited by a special apparatus at the end of the tube, as has been pointed out in a recent paper (4). With ordinary driving apparatus, therefore, such as was used in the previous experiments, this vibration occurred only when the experimental tube had a thin wall.

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* *Ref. (2), Equation 31.*

Conclusions

For a fluid contained in a tube having a wall either thick or of a material having a high acoustic resistance compared with that of the fluid, the longitudinal wave that will be produced in the fluid by an ordinary driving apparatus at one end of the tube will be plane. If, however, the wall is essentially nonrigid, the longitudinal wave will not be plane, strong radial vibrations will occur, and as a result the longitudinal wave will progress only with difficulty when its frequency is near to that of the resonant radial vibration of the column of fluid.

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MEASUREMENT OF SMALL OPTICAL ACTIVITIES WITH THE QUARTZ CRYSTAL LIGHT MODULATOR[1]

By D. W. R. McKinley[2]

Abstract

An experimental procedure is described for using the quartz crystal light modulator as an instrument for measuring very small optical rotations of the plane of polarization. The accuracy of the measurement is of the order of 10 sec. of arc, and the method is capable of still greater precision.

An elementary analysis of the crystal behaviour explains the fundamental and second harmonic components of light intensity present in the modulated beam of light.

In a previous paper (2) it was pointed out that optimum light modulation effects occur when light is passed through a vibrating quartz crystal in the direction of the optical axis. A special 49°-cut* crystal was adopted, because it satisfied this requirement in addition to possessing several other advantageous features. In this report some further experiments are described, an attempt is made to explain the results by an elementary analysis of the crystal action, and the technique is applied to the measurement of small optical activities.

The experiment may be arranged as shown in Fig. 1. Light from a pointolite source is polarized by the Nicol prism, N_1, (in most of this work Polaroids have been substituted for the Nicols with equal success) and is then focused on the crystal aperture by means of the lens, L_1. The crystal is set at the focus of a second lens, L_2, and is so inclined that the beam of light passes through nearly parallel to the optic axis. The light emerging from L_2 will traverse any intervening medium, X, in an approximately parallel beam. After passage through a second Nicol, N_2, the light is finally focused on a high vacuum photocell, P.

The photocell circuit, C, may be tuned either to the fundamental frequency of the crystal or to twice that frequency. The fundamental frequencies of the crystals used in these experiments were in the neighbourhood of 4 megacycles per second, and the crystals were excited at their fundamental frequencies only. The amplifier-detector system following the photocell comprises, in effect, the radio-frequency, intermediate frequency, and second detector

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Contribution from the McLennan Laboratory, Department of Physics, University of Toronto, Toronto, Canada. Part of this work was undertaken at the University of Toronto as an assisted research under the National Research Council of Canada.

[2] *Physicist, National Research Laboratories, Ottawa, Canada.*

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* *W. G. Cady has suggested that the convention regarding the orientation of the crystallographic axes in quartz be given, since the system used by Lack in his work on these crystals differs from that employed by Voigt. Lack et al. (1) state that for a rotation about the electric axis a positive angle is that formed by a clockwise rotation of the optic axis of the crystal when the electrically positive face (determined by a squeeze) is up. For a left-handed crystal a positive angle is in a counterclockwise direction. The inclination of the optic axis to the face of the crystals used in these experiments is minus 49° by this convention.*

sections of a standard high-frequency superheterodyne radio receiver. The milliammeter and galvanometer are placed in the plate circuit of a vacuum tube voltmeter connected across the resistor of the diode detector.

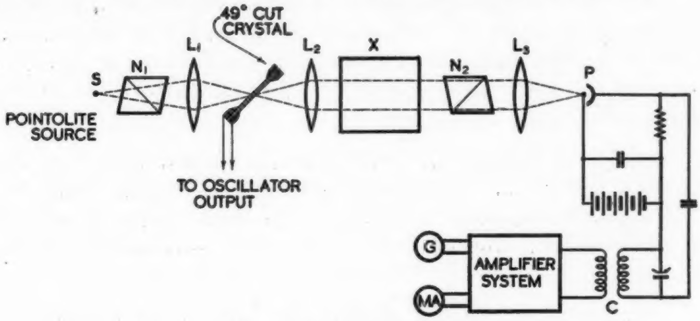

FIG. 1. *Schematic diagram.*

Let us first consider the arrangement of Fig. 1 but with no optically active medium at X. The principal plane of the first Nicol should always be set so that the light vibrations are executed in the plane of incidence, referred to the crystal faces. Then N_2 is rotated until the light intensity becomes a minimum, which will occur when the Nicols are nearly perpendicular. If the crystal is now excited at its fundamental frequency, light passes through N_2 in flashes. These light flashes falling on the photocell cause an alternating voltage to be developed across the circuit C. The galvanometer readings are proportional to this voltage.

In the preliminary experiments the second Nicol was rotated and the galvanometer readings were taken for the various settings of N_2. One set of observations was obtained in this manner with the photocell tuned to the crystal frequency, and another set with the photocell tuned to twice the crystal frequency. No galvanometer indications could be observed with the photocell circuit tuned to any other frequency, including higher harmonics of the crystal frequency. In all cases, of course, the crystal was driven at its fundamental frequency only.

In Fig. 2 the dotted curve represents the first case when the photocell was tuned to the fundamental, and the solid curve shows the results obtained with the photocell tuned to the second harmonic. There was not inconsiderable difficulty encountered in adequately shielding the receiving apparatus from direct electromagnetic pick-up from the crystal oscillator when the receiver was tuned to the crystal frequency. As a consequence the dotted curve shown does not give a reliable indication of the amplitudes, but it is useful in determining positions of zero response. However, it was possible to shield the equipment sufficiently well to eliminate all trace of stray pick-up when the receiver was tuned to the second harmonic. As will be shown, the theoretical considerations indicate that either fundamental or second harmonic operation should be equally useful, but in practice the latter is greatly to be preferrred.

From a consideration of the solid curve, it is seen that, if the vacuum tube voltmeter reading observed when the Nicols are crossed is represented by V, then that obtained with the Nicols parallel is slightly higher, or about 1.25 V. Consequently, for certain applications in which maximum intensity of the modulated light is important, the crystal system should be operated with the Nicols in the parallel position. This practice was followed in an experiment on the velocity of light carried out at the McLennan Laboratory, University of Toronto.

If N_2 is removed entirely, the voltage across the photocell drops to about 0.8 V, and if both Nicols are removed the resultant response drops to about 0.1V, as might be expected since the crystal is now behaving merely as a diffracting body, with additional polarizing effects produced at the inclined faces.

As N_2 is rotated, the phase of the modulated light beam suddenly changes by 180° at each null point. This was easily demonstrated by connecting one pair of plates of a cathode ray oscillograph across the second detector input, and applying some voltage directly from the crystal oscillator to the other pair of plates. The resultant pattern showed that the phase reversed at each null point of the curves of Fig. 2.

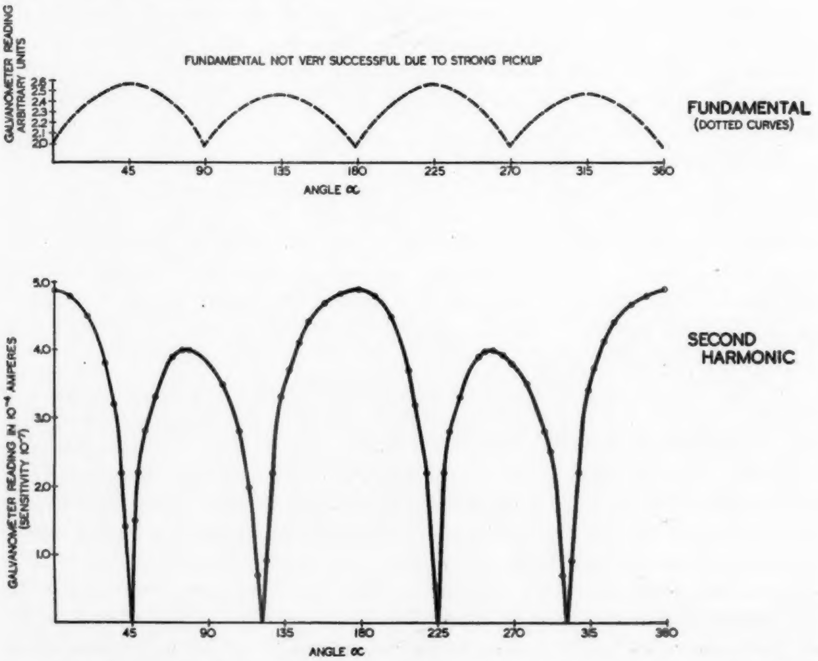

FIG. 2. *Curves showing galvanometer readings plotted against the angle α between the principal planes of the polarizing and analyzing Nicols.*

The action of a vibrating medium on a beam of light passing through it may be adequately explained in the general case by the theory of diffraction. However, it seems that in the particular case where light is passing along the optic axis, the optical activity of the quartz masks the diffraction patterns normally predicted. In fact, the optical activity here appears to play the more important part in the modulation of light, and the analysis of the experimental results is simplified accordingly.

Suppose the assumption is made that the plane of polarization of the light beam as it emerges from the crystal is dynamically rotated about its initial position, and that the light remains plane polarized to a large extent. Then the periodic angular change of the effective plane of polarization may be represented by $\theta_0 \sin \omega t$, where $\omega = 2\pi f$ and $f =$ the crystal frequency. The intensity of light transmitted through N_2 will be,

$$I = I_0 \cos^2(\alpha - \theta_0 \sin \omega t) \tag{1}$$

where α is the angle between N_2 and N_1, N_1 remaining fixed. That is,

$$\begin{aligned} I &= \frac{I_0}{2}\Big[1 + \cos 2(\alpha - \theta_0 \sin \omega t)\Big] \\ &= \frac{I_0}{2}\Big[1 + \cos 2\alpha \cos (2\theta_0 \sin \omega t) + \sin 2\alpha \sin (2\theta_0 \sin \omega t)\Big] \end{aligned} \tag{2}$$

When the crystal is being operated within safe limits the amplitude of the dynamic rotation may be assumed to be small. Then, for $2\theta_0 < \frac{\pi}{2}$ the term, $\sin 2\alpha \sin (2\theta_0 \sin \omega t)$, represents an alternating light intensity of amplitude $\sin 2\alpha$ and effective frequency $\frac{\omega}{2\pi}$. The term $\cos 2\alpha \cos (2\theta_0 \sin \omega t)$ represents an alternating component of amplitude $\cos 2\alpha$ and effective frequency $\frac{\omega}{\pi}$. This follows by considering that the cosine of a negative angle between 0 and $-\frac{\pi}{2}$ remains positive, so that the cosine term has two maxima and two minima per period of the crystal, $T = \frac{2\pi}{\omega}$, whereas the sine term has but one maximum and one minimum in the same time interval.

For a given value of α the receiving system averages the oscillating value of the light intensity. With the photocell tuned to frequency $\frac{\omega}{2\pi}$ the component $\sin (2\theta_0 \sin \omega t)$ alone will affect the detector. Let its average value be A_1. With the system tuned to the second harmonic the term $\cos(2\theta_0 \sin \omega t)$ alone will be averaged, with value A_2, say. The amplitude of these average values is determined by the factor containing α, so the two cases may be written,

$$I_1 = A_1 |\sin 2\alpha| \tag{3}$$

for the fundamental frequency, and,

$$I_2 = A_2 |\cos 2\alpha| \tag{4}$$

for the second harmonic frequency, and in each case the absolute value is written because of the phase change of π at the null points; the light intensity never becomes negative.

Equation (3) corresponds to the dotted curve of Fig. 2, and Equation (4) to the solid curve. It will be noticed that the zeros of modulated light intensity on the solid curve vary slightly from the 135° and 315° positions of N_2. This is due to the statical rotation of the plane of polarization in the quartz slab, which has an effective optical thickness of 0.8 mm., so that the average statical rotation for white light is around 12° to 15°. The deeper modulation obtainable for the values of 0° and 180° might possibly be explained by the lessened colour absorption of the Polaroids in the parallel position, though the exact reason is not immediately apparent.

Applications

In some investigations it is desirable to have two beams of modulated light of the same frequency but with a phase difference of π. This has usually been accomplished by dividing the beam after it has passed through N_2 and permitting one of the resultant beams to traverse a path which is one-half wave-length longer than the other (at the modulation frequency, not the actual frequency, of the light). Equation (3), or Equation (4), suggests that two such beams may be obtained by dividing the beam immediately after it emerges from the crystal and then placing an analyzing Polaroid in each of the beams, one Polaroid being parallel to the polarizer and the other perpendicular to it.

Another and more interesting application is suggested by Equation (3) in the neighbourhood of 0°, 90°, 180°, and 270°; or by Equation (4) near 45°, 135°, 225°, or 315°. In the latter case the empirical values are 45.0°, 122.5°, 225.0°, and 302.5°. Near these points the curves drop very steeply, and a small angular rotation of N_2 produces a large change in the photocell response. With the radio receiver available in this research a change in the galvanometer reading of 1×10^{-7} amp. could be easily detected. As the maxima are of the order of 5×10^{-4} amp., this corresponds to an accuracy of 1 part in 5000 over a range of 45°. If the total angular variation is small one can work on the steep portion of the curve where the accuracy is much greater; for example, the error is 1 part in 2500 for a range of 5°, if the 5° are covered near one of the null points, and this corresponds to an angular error of only 8 to 10 sec. of arc. Thus the arrangement provides a sensitive device for measuring small optical rotations in any other medium which may be placed at X in Fig. 1.

Sugar solutions were made up to test the accuracy of the apparatus. The experimental results agreed within 20 sec. of the calculated values for solutions whose average rotatory power for white light ranged from 1.5 min. to 2°. Two methods of obtaining the experimental values were followed. In the first, the galvanometer curve was calibrated in terms of the angle change of the second Polaroid, which was mounted on a micrometer goniometer table, and the galvanometer readings were observed before and after addition of sugar to the water cell. In the second method, the position of N_2 for zero response was noted, then the sugar solution cell was inserted and the new position of N_2 for zero response was observed.

The first method incurs the smaller error because the instrument is operating entirely on the steep portion of the curve, and this procedure is to be preferred in investigations of the Faraday effect, for example, in which the medium is always present at X and no change occurs in the absorption of light. If the medium under test is strongly absorbing and if a correction for the absorption cannot be readily determined, then the second method should be employed, though it is somewhat less accurate because the actual minimum is not a perfect cusp, but is slightly rounded.

Acknowledgments

This work was carried out under the direction of Dr. E. F. Burton. The author also wishes to acknowledge the advice and assistance of Dr. H. A. McTaggart, Dr. M. F. Crawford, and Mr. A. Pitt.

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FRACTIONATION OF THE CHLOROFORM EXTRACT OF MAPLE SYRUP[1]

BY LOUIS SAIR[2] AND J. F. SNELL[3]

Abstract

A method of fractionating the chloroform soluble constituents of maple syrup has been devised. Marked differences were observed among Quebec syrups of different years. Fat constituted half the weight of the extract of the 1935 syrup but was completely absent in the 1936, and present in very small quantity in the 1937, product. Crystals of carbonyl compounds having vanillin odour were isolated from bisulphite fractions of the 1935 and 1936 extracts, but the crystals from the two years' syrups differed from each other in melting point and chemical behaviour. Vanillin was not found. An odourless fraction constituting 35% in 1935 and 65% in 1936 had a composition and a behaviour similar to those of lignin. The substance chiefly responsible for maple odour is indicated to be an enolic viscous oil, volatile at 0.03 mm., and present in the 1936 and 1937 syrups in the proportions of 0.6 gm. per 100 gal. of syrup, or about 1 p.p.m.

Introduction

The characteristic flavouring principle of maple sap syrup has not been definitely isolated. Robison (19) found that the flavour could be extracted from the syrup or from maple sugar by ether, chloroform, or benzene. With saturated sodium bisulphite solution, Nelson (17) and Skazin (22) separated vanillin-like products from ether and chloroform extracts, respectively, but the former concluded that maple flavour was due rather to a phenolic than to this carbonylic material. Skazin (22) demonstrated that the flavour is developed in the boiling process. Findlay (8) and Risi and Labrie (20) confirmed this conclusion. The former attempted to discover in the raw sap the substance from which the flavour is produced and asserted a relation of this precursor to ferulaldehyde and to lignin. Labrie (14) and Risi and Labrie (20) attributed the flavour to Czapek's "hadromal" (4, 5, 10), a material whose standing as a chemical compound is not established (9, 18). They preceded Findlay in suggesting a relation to lignin and claimed to have synthesized the flavouring principle from guaiacol, vanillin, and furfural (nascent from sucrose).

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[1] *Manuscript received in original form November 18, 1938, and as revised, May 27, 1939. Contribution from the Chemistry Department, Macdonald College (McGill University), Quebec, Canada. Presented in preliminary form at the Canadian Chemical Convention, Vancouver, B.C., June 1937. Macdonald College Journal Series No. 106.*

[2] *Formerly Research Assistant. This paper is based on a thesis accepted in partial fulfilment of the requirements of McGill University for the degree of Ph.D. The work on the 1937 syrup was done in the Laboratories of the National Research Council, Ottawa, after attainment of the degree.*

[3] *Emeritus Professor of Chemistry.*

None of these investigators developed a satisfactory method of fractionating the ether or chloroform extract. The writers have endeavoured to devise a method which would accomplish fractionation without destroying the maple flavour and have made a partial study of some of the products so obtained. These include a fat, two crystalline carbonyl products, bearing some resemblance to those found by Nelson and Skazin, five amorphous lignin-like products and a neutral oil, volatile at 0.02 mm., which develops maple odour on standing, and which the results indicate to be the essential flavouring principle.

Experimental

Material

The Quebec Department of Agriculture, then presided over by the Hon. Dr. J. Adelard Godbout, generously donated maple syrup of good flavour and medium colour collected by "Les Producteurs de Sucre d'Érable de Québec" in the seasons of 1935 and 1936. It was delivered in one-gallon containers as received from individual farmers. As will appear, the extracts made from the syrups of the two seasons showed surprising differences in composition.

Extraction

Two-gallon portions of syrup were diluted with three-fourths their volume of water and shaken with chloroform in a glass bottle enclosed in a barrel churn. Each portion was shaken for five hours with 1500 cc. of chloroform and for a like period with another 1500 cc. The combined extract was concentrated at atmospheric pressure to a volume of approximately 200 cc. and was kept in a refrigerator. It was later converted by vacuum distillation to a reddish, viscous residue having a decided maple odour.

Fractionation

The viscous residue was taken up in 95% alcohol. From the alcohol solution pertaining to each lot of 1935 syrup, whatever its place of origin, a soft solid (Fraction *A*) separated on cooling. This was a fat (see below). It constituted about half the chloroform extract. No corresponding product was found in any of the 1936 syrups.

After removal of *A*, the alcohol was evaporated *in vacuo* and the residue was dissolved in chloroform, and shaken in an ice bath with two successive 50 cc. portions of saturated sodium bisulphite solution. The material thus extracted constitutes Fraction *B* (see flowsheet).

The exhausted, red, chloroform solution was dried with sodium sulphate and evaporated *in vacuo*. The residue, a viscous oil, when subjected to the higher vacuum of a Hyvac pump, swelled, filling the flask, and leaving an amorphous residue, which was ground to a scarlet powder of distinct maple odour. Qualitative tests indicated the presence of phenols, acids, carbonyl compounds, and neutral products.

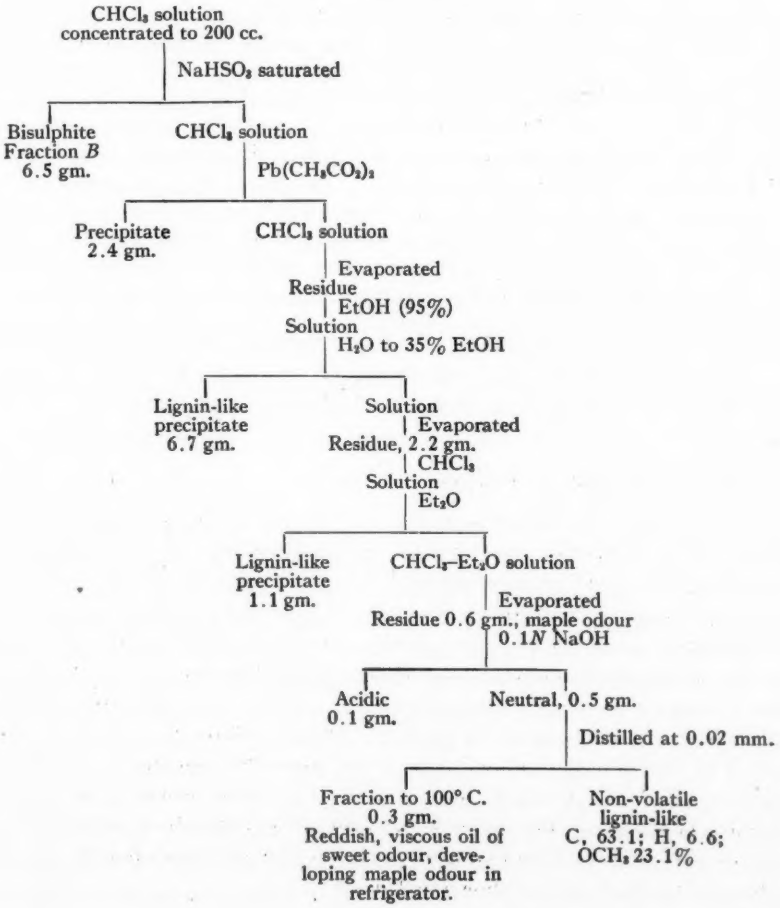
The procedure finally adopted for further fractionation is shown in the flowsheet. This procedure was adopted in the light of preliminary attempts to separate the constituents by use of organic solvents, mild alkalies, and water. These preliminary experiments yielded the following information:

1. Petroleum ether dissolved only 5% of the residue. Ethyl ether (peroxide-free), applied to the remainder, dissolved 45% of the original. The remaining 50% was still soluble in chloroform. Only the ethyl ether extract had maple odour.

FLOWSHEET

CHLOROFORM EXTRACT OF MAPLE SYRUP (50 GAL., 1936)

Final method of fractionation


$CHCl_3$ solution concentrated to 200 cc.
$NaHSO_3$ saturated
Bisulphite Fraction *B* 6.5 gm.
$CHCl_3$ solution
$Pb(CH_3CO_2)_2$
Precipitate 2.4 gm.
$CHCl_3$ solution
Evaporated
Residue
EtOH (95%)
Solution
H_2O to 35% EtOH
Lignin-like precipitate 6.7 gm.
Solution
Evaporated
Residue, 2.2 gm.
$CHCl_3$
Solution
Et_2O
Lignin-like precipitate 1.1 gm.
$CHCl_3$–Et_2O solution
Evaporated
Residue 0.6 gm., maple odour
0.1*N* NaOH
Acidic 0.1 gm.
Neutral, 0.5 gm.
Distilled at 0.02 mm.
Fraction to 100° C. 0.3 gm. Reddish, viscous oil of sweet odour, developing maple odour in refrigerator.
Non-volatile lignin-like C, 63.1; H, 6.6; OCH_3 23.1%

2. Sodium bicarbonate (5%), ammonium hydroxide (1 : 10), and sodium hydroxide (2%) applied in succession to the chloroform solution of the ether extract, removed, respectively, 7, 10 and 57% of the total, leaving a neutral fraction of 25%, a red mixture of oil and solid having a pleasant odour. The alkaline extracts were odourless.

The results with ammonium hydroxide are in contrast to Nelson's experience with direct ether extracts of maple syrups (17). In many tests on 1935 and 1936 syrups, ammonium hydroxide was found to extract only a minor portion of the ether extract and to destroy the odour.

3. The ether-insoluble portion of the extract and the alkaline extracts of the ether solution are similar products—acidic, lignin-like and odourless (see below, Product *C*).
4. Water dissolves the maple odour material and yields it up again to chloroform. The aqueous extract can be separated into (i) neutral, (ii) phenolic, and (iii) carboxylic fractions by (i) titrating to pH 8.1 with 0.1 *N* sodium hydroxide, (ii) saturating with carbon dioxide, and (iii) acidifying, shaking out with chloroform after each operation. The neutral fraction constitutes about five-sixths of the whole and carries the odour, though modified. The phenolic fraction is odourless, the carboxylic slightly rancid. The neutral fraction reduces Fehling's solution and answers to tests for the carbonyl group but not to the Millon and Liebermann tests for phenols. The portion distilling below 140° at 0.02 mm. (30% of the total) develops true maple flavour in the refrigerator.
5. Lead acetate, which Findlay (8) found would precipitate a portion of the original chloroform solution, precipitates the higher distilling portions of the neutral fraction but not that coming over below 140° C.

Products

A. The Alcohol-insoluble Fraction

This fraction constituted half the total weight of the chloroform extract of the 1935 syrup but was entirely absent from that of the 1936 season. It is a yellow saponifiable oil, probably identical with that which Nelson (17) found in Vermont, but not in Michigan, syrup, and which he attributed to fat added to the sap to prevent foaming. In the writers' work, trials on lots of one, two, four, or more gallons from individual producers invariably gave this product, which must therefore be regarded as a normal constituent of the sap of the 1935 season in Quebec. It had a refractive index of 1.457 at 20° C., Hübl iodine number, 41 to 43; saponification number, 270; acid number, zero; unsaponifiable matter, 1.9% (a yellow oil with a penetrating, shellac-like odour, giving negative Liebermann and Whitby tests for sterols). The properties of the liberated fatty acids from 15 gm., separated by the lead-salt-ether method, were as shown in Table I.

TABLE I

PROPERTIES OF LIBERATED FATTY ACIDS

	Weight	Hübl value	Mol. wt. by titration
Saturated	6.4	6–7	261
Unsaturated	5.6	88–90	275

Fractionation of the saturated acids according to Rosenthaler (21) yielded (i) 1.05 gm. of m.p. 62 to 64° C.* and mol. wt. 261, (ii) 1.40 gm. of m.p. 60 to 61° C., mol. wt. 256; (iii) 1.20 gm. of m.p. 57 to 61°, mol. wt. 254; and a fourth fraction (soluble in 50 cc. absolute alcohol at 0°), which was lost. Recrystallization of Fraction (i) yielded no higher melting product. The results point to palmitic acid (m.p. 62.6, mol. wt. 256) as the predominating acid. Myristic acid may be present in very small proportion. The unsaturated acids must be mainly oleic (iodine number, 90; mol. wt., 282). Treatment with bromine in acetic acid yielded no crystalline product. Glycerol was detected by the acrolein test and by three colour tests described by Morrow (16, p. 238).

B. The Bisulphite Fraction

Nelson (17) and Skazin (22), using bisulphite extraction, and Labrie (14), extracting an ether extract with petroleum ether, obtained evidence of the presence of substances resembling vanillin. None of them succeeded in satisfactorily identifying this aldehydic material. Not only did their opinions as to its nature differ, but Nelson obtained different results on the two syrups he examined. The writers' experience is similar. The bisulphite fractions from the syrups of 1935 and 1936 differ so radically as to require separate description.

1935

The use of acids to decompose the bisulphite addition compound was avoided, sodium carbonate being used instead. To the bisulphite solution and 200 cc. of chloroform in a separatory funnel a 10% solution of sodium carbonate was added slowly in 10 cc. portions without shaking. The process was repeated several times with fresh portions of chloroform, the object being to limit the time of exposure of the odorous substance to the action of the alkali. The different extracts were combined, dried with sodium sulphate, concentrated to 200 cc. and taken to dryness *in vacuo*. From 30 gal. of syrup, 0.65 gm. of a yellow, viscous oil, with an odour like that of vanillin, was obtained. The mass was extracted with boiling petroleum ether. Evaporation of the ether yielded a few crystals, contaminated with a yellow oil. Attempts to free the crystals from this oil by use of activated carbon, or of solvents, simple or mixed, failed. The crystals were very unstable and, when left standing in the air or exposed to heat, quickly changed to a reddish oil. Similar results had previously been obtained in this laboratory by Skazin (22), who followed Nelson's procedure of decomposing the bisulphite compound with dilute sulphuric acid. The crystalline product was finally isolated by extracting the chloroform solution of the bisulphite fraction with ammonium hydroxide (1 : 10), acidifying, extracting with chloroform, and evaporating *in vacuo*. Silvery white crystals of m.p. 90 to 92° C. were obtained (14 mg. from 10 gal. of syrup). This product reduced Fehling's solution, decolorized bromine water, and gave a brown colour with ferric chloride. A 2,4-dinitrophenylhydrazone, prepared according to the method of Allen (1), showed m.p. 215 to 217° C. and OCH_3, 12.7%. This product is obviously not vanillin. None of it was found in the 1936 syrup.

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* *Melting points are uncorrected.*

1936

Treatment of the chloroform extract of this syrup with sodium bisulphite yielded a red, gummy precipitate (3.5 gm. from 30 gal. of syrup). This is in contrast to the behaviour of the 1935 syrup, which yielded no insoluble addition product.

The bisulphite soluble fraction (and the washings from the precipitate), decomposed by sodium carbonate, yielded a viscous, yellow, vanillin-smelling oil (0.5 gm. from 30 gal. of syrup). As previously stated, it was not found possible to isolate a crystalline product from this oil by the method of extraction by ammonia used in 1935. The oily material was phenolic in behaviour, in that it was soluble in ammonium hydroxide solution and completely precipitated by carbon dioxide. The use of organic solvents also proved ineffective. However, distillation at 0.03 mm. pressure from a 160° C. oil bath yielded 40% of volatile material, which, when resublimed at a bath temperature of 80 to 90°, consisted of a white crystalline material mixed with oil. By dissolving in 0.5 cc. of chloroform, adding petroleum ether until turbid, cooling in the refrigerator, and repeating this procedure 10 to 15 times, almost colourless (very faintly yellow) crystals, melting at 110 to 112° C. and having a vanillin odour, were obtained in the proportion of 45 mg. per 50 gal. of syrup. Analysis showed—C, 57.3; H, 5.5; $OCH_3 + OC_2H_5$, 30.3%. Calcd. for $C_9H_6O_4(OCH_3)(OC_2H_5)$: C, 56.7; H, 5.5; $OCH_3 + OC_2H_5$, 29.8%. A 2, 4-dinitrophenylhydrazone was obtained as fine, long, scarlet needles, m.p. 232 to 233° C.; OCH_3, 12.4%. Calcd. for two carbonyl groups, 12.3%.

The crystalline product gave a positive xanthate test for hydroxyl, positive iodoform and nitroprusside tests for methyl ketone structure, and negative Fehling, Schiff, Liebermann, and Millon tests. A crystalline acetate could not be obtained. The behaviour indicates a benzene derivative with one alcoholic and one phenolic hydroxyl, two carbonyl groups (one of which is methyl ketonic), a methoxyl, and an ethoxyl group. This would account for the six oxygen atoms.

The study of the bisulphite fraction clearly indicates the absence of vanillin from the 1935 and 1936 Quebec syrups, and confirms the conclusion of previous investigators that the substances of vanillin-like odour and carbonylic constitution are not the *chief* contributors to the flavour of maple products.

C. The Lignin-like Fraction

This fraction constituted 60 to 70% of the total chloroform extract of the 1936, and 35 to 40% of that of the 1935, syrup. It was red, amorphous, and odourless. The writers' observations yielded no indication of a relation to the flavour of the syrup. With the successive use of barium hydroxide, hydrochloric acid, sodium hydroxide, and carbon dioxide it was separated into five portions. Four of these had elementary compositions (C, 59.0–62.6; H, 6.4–6.7; OCH_3, 19.6–21.4%) within the range of recorded analyses of lignin (3, 11, 13). The chemical behaviour also corresponded to that of lignin. The fifth portion, constituting about one-eighth of the whole, had the composition—C, 46.9; H, 6.1, OCH_3, 19.2%. It was more highly coloured than the others and resistant to acid hydrolysis.

The presence of "free", soluble lignin in wood has been reported by von Euler (6), Klason (12), and Wislicenus (23). The last-mentioned found appreciable quantities in the sap of the sugar maple.

D. The Maple Odour Fraction

The product which, in the writers' opinion, is chiefly responsible for maple odour is the neutral volatile viscous oil (see flowsheet). The true maple odour was modified to a sweet one either by the unavoidable treatment with 0.1 *N* alkali or by the distillation, but it was restored on standing a few days in a refrigerator.

This component was isolated from both the 1936 and the 1937 syrup. Results of analyses were as shown in Table II.

TABLE II

ANALYSES

	C	H	O (by diff.)	OCH_3
Product of 1936	70.2	9.3	30.5	10.2
Calcd. for $C_{18}H_{28}O_4$	70.1	9.1	20.8	10.1
Product of 1937	66.14	7.87	25.99	8.93
Calcd. for $C_{20}H_{28}O_6$	65.93	7.69	26.38	8.52

The 1937 analysis refers to a product that was redistilled and was therefore considered purer than that of 1936.

The following properties of the 1936 product were observed: it has refractive index of 1.555 at 20° C.; is optically inactive; reduces Fehling's solution; decolorizes bromine water; gives negative tests (xanthate, acetic anhydride) for alcoholic, and negative tests (Millon, Liebermann) for phenolic, hydroxyl; gives negative tests (iodoform, nitro-prusside) for methyl ketone, and for ester, structure [Feigl, (7)]; it gives positive tests for carbonyl group [precipitate with 2,4-dinitro-phenylhydrazine, and [Kurt-Meyer (15, pp. 325-326)] for enolic hydrogen (15.0% by iodimetry)].

Attempts to prepare crystalline products by oxidation with 1 : 4 nitric acid or with neutral permanganate and by reduction with powdered zinc failed, as did also attempts to produce oximes, semicarbazones, and acetyl derivates. The 2,4-dinitrophenylhydrazone obtained was amorphous and of low yield and indefinite melting point.

As the combustion analysis suggested a possible relation to phenanthrene, the bulk of the 1937 product was reduced by the Clemmensen method and the reduction product dehydrogenated with selenium. A high-vacuum distillation of the dehydrogenated material yielded a white crystalline product which was very deliquescent and was insoluble in non-hydrolyzing organic solvents. An analyst in Germany to whom this sublimate was submitted for combustion reported that it contained only traces of organic matter. It was found to be selenium-free and would appear to have been zinc chloride.

To make further study of the chemical nature of the odorous oil would have involved repeating the fractionation with a further supply of maple syrup. This the writers were not in position to do.

Discussion

In describing the products the writers have referred in the main to maple *odour* rather than to maple flavour, since in identification they have relied on smell rather than on taste.

Although the data obtained do not lead to a clear knowledge of the chemical nature of the odorous principle of maple syrup, it is the writers' hope that their method of separating the constituents of the chloroform extract and the isolation, from syrups of two seasons, of a product (or closely related products) in which the characteristic odour develops on standing, may be found of value in future investigation of this problem, to which, in the writers' opinion, no satisfactory solution has yet been found.

Acknowledgments

The writers have to thank Dr. W. D. McFarlane of Macdonald College and Dr. Richard H. Manske of the Chemistry Division of the National Research Laboratories, Ottawa, for their interest and helpful advice.

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THE PREPARATION OF ETHERS[1]

By Philip G. Stevens[2] and Sidney A. V. Deans[3]

Abstract

A new modification of Williamson's ether synthesis, using sodium naphthalene to form the metal alcoholate, has been described, by which the yields and ease of preparation of ethers have been improved.

The most general method for the preparation of ethers from carbinols is still that developed by Williamson (11) over eighty years ago. This consists in treating a metal alcoholate with an alkyl halide, and is the only method which permits the preparation of optically active ethers with the certainty that no Walden Inversion can occur. This method, as usually carried out, has many disadvantages however. One of these is that the yields are seldom greater than 50 to 60% (3), probably due to the fact that the alcoholate formed becomes associated with one or more molecules of the alcohol, which then does not react with the metal (10). This results not only in poor yields, but also in contamination of the ether with the alcohol, necessitating its removal either by repeated distillation from sodium, or by treatment with phthalic anhydride. Another disadvantage is that the higher molecular weight alcohols react even with potassium so sluggishly that often heat must be applied. This sometimes causes racemization as well as the formation of complex products, if two hydrogen atoms are in the α- and β-position to the oxygen atom, as shown by Hückel and Naab (2) with *cis* decalol, and confirmed later by Stevens (9) with methylisopropylcarbinol and 2-ethoxy-3-methyl-butanol-3. A third disadvantage is that the metal may attack the rest of the molecule. To avoid this, Purdie and Irvine (7) used silver oxide and methyl iodide for the methylation of carbohydrates. More recently Kraus and White (4) carried out methylations in true Williamson style by using sodium or potassium in liquid ammonia; and this modification has later been extended to carbohydrates with excellent yields (5). However this method is effectively applicable only with those alcohols or polyhydroxy compounds which are soluble in liquid ammonia, and which form soluble metal salts, and it fails for simple alcohols like hexanol-2.

The writers have now found that almost any alcohol can be very easily converted to its methyl ether with the aid of sodium naphthalene, recently described by Scott, Walker and Hansley (8), as a reagent ideally adapted for the preparation of sodium derivatives of alcohols or other compounds with active hydrogen. The yields are extremely good, and optically active alcohols yield ethers of high rotatory power. The method consists in adding the alcohol to the sodium naphthalene dissolved in an "effective" ether such as glycol dimethyl ether. The formation of the alcoholate is quantitative, and the exact end of the reaction is indicated by the sharp change in colour of the solution. Addition of methyl iodide or methyl sulphate completes the reaction, the products of which are then worked up in the usual way. The only important point is to choose reagents which will not interfere with the isolation of the ether. This is easily accomplished by the use of the proper "effective" ether and aromatic hydrocarbon (8)*. Thus with hexanol-2 and cholesterol, naphthalene is satisfactory, since it can be easily separated from each ether by fractional distillation and steam distillation respectively. With linalool, diphenyl with its higher boiling point is preferred.

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The lower molecular weight alcohols gave ethers in less satisfactory yields simply because of manipulative losses and inadequately effective fractionating columns. In these cases the use of dimethyl ether as the solvent might be better, since it is difficult by distillation to separate the methylvinyl ether (b.p. 12 to 14° C.) which is formed by cleavage of the glycol ether (8). Hydroxy compounds with other functional groups, such as ethyl lactate, can also be converted to ethers in this way by merely reversing the process of addition. Here the yields are again lower, but still about as good as those reported by Purdie and Irvine (6) who used silver oxide and methyl iodide.

Experimental

Sodium naphthalene (or diphenyl) is first prepared in glycol dimethyl ether solution according to the directions of Scott, Walker, and Hansley (8). This intensely coloured solution is cooled, and then the alcohol to be etherified is added with mechanical stirring. The sharp colour change from deep green (or blue) to a pale greenish yellow may appear before one equivalent of the alcohol has been added, if some sodium remains undissolved. The addition is then stopped at this point, and the stirring continued until the sodium has practically all dissolved. The titration is then continued until the deep colour again disappears. This occurs now when one equivalent of the alcohol has been added. Either methyl iodide or sulphate is added slowly, keeping the solution below 20° C., and the mixture is allowed to stand overnight.

If the ether is a low boiling one, methyl sulphate is used, and the products can be fractionally distilled directly. If the ether is a high boiling one, methyl iodide is used, the reaction mixture treated with water, and the product extracted with ether, dried, and distilled. In the case of cholesterol, it is necessary to use considerably more glycol ether to maintain a homogeneous solution. After the reaction is over, the entire mixture is steam distilled to remove the naphthalene along with the more volatile materials. The sterol ether is far less volatile than naphthalene, and on cooling, sets to a cake, and is recrystallized from acetone. With ethyl lactate, the sodium naphthalene must be added to the ester to avoid, as much as possible, interaction of excess sodium naphthalene with the carbonyl group. In this case, as no change of colour indicates the end-point, stoichiometric amounts of each reagent are used, methyl iodide is added, and the products worked up in the usual way. In Table I are assembled all the experimental data.

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* *The authors' sample of commercial anthracene formed a sodium derivative, but this did not undergo the proper colour change when treated with linalool. This may be due to impurities in the anthracene used.*

TABLE I

Alcohol	α_D^{23}	Methyl ether α_D^{23}	B.p., °C.	Yield, %	Reagents used
Propanol-2	—	—	31 (752 mm.)	51	Naphthalene, methyl sulphate
2-Methyl-propanol-2	—	—	54-6 (762 mm.)	55	Naphthalene, methyl sulphate
Hexanol-2	+ 7.1	+ 7.6	115–7 (754 mm.)**	91.5	Naphthalene, methyl iodide
Linalool	−15.2	+14.8	72–5 (10 mm.)†	91.3	Diphenyl, methyl iodide
Cholesterol	—	—	M.p. 83–4	87	Naphthalene, methyl iodide
Ethyl lactate	− 6.8	−49.6	139–43 (761 mm.)	60	Naphthalene, methyl iodide

** *This ether after distillation from sodium had the following constants: b.p. 115 to 116° (763 mm.); n_D^{25} 1.3916; d_4^{25} 0.7685. The methoxyl content was 3.5% too high.*

† *On redistillation at 758 mm. the linalool ether boiled at 193.5 to 194°; α_D^{23} + 14.3°; n_D^{25} 1.4481* [*Compare Barbier* (*1*)].

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CALYCANTHINE

IV. A STRUCTURAL FORMULA[1]

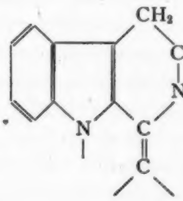
By Richard H. F. Manske[2] and Léo Marion[2]

Abstract

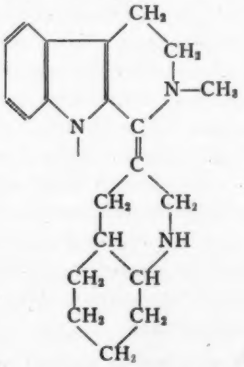
The alkaloid calycanthine ($C_{22}H_{26}N_4$), when degraded with selenium, yields a mixture of norharman, lepidine, skatole, β-ethyl-indole and a base. Heating calycanthine with palladium liberates ammonia. Benzoylation followed by oxidation brings about scission of the molecule into benzoyl-N-methyl-tryptamine and an acidic fragment which contains a benzoylated nitrogen, and another nitrogen, presumably included in a ring. A chemical structure which will account for these results is suggested. On the basis of the suggested formula, the foregoing acidic fragment should contain a hydroquinoline nucleus and it should be possible to dehydrogenate it to quinoline. Such is found to be the case. The formula suggested by Barger, Madinaveitia, and Streuli (1) is discussed.

The alkaloid calycanthine, since its definite characterization by Gordin (2), has been the subject of a number of communications. The most recent is that of Barger, Madinaveitia, and Streuli (1), which contains a structural formula for the alkaloid. We have had this problem under study for several years and are now in a position to suggest for calycanthine a structural formula which differs radically from that published by the authors mentioned above (1).

One of us has shown that N-methyl-tryptamine is obtainable from calycanthine by comparatively mild treatment and has advanced a partial structural formula, (I), representing the carboline-half of the molecule (7). The isolation of quinoline from calycanthine by treatment with hydriodic acid and phosphorus has already been reported by us (8). Now it has been possible to obtain quinoline by an entirely different process, as will be discussed later in this paper. We, therefore, do not believe that quinoline is obtained as a result of deep-seated rearrangements, but that the quinoline nucleus is present in the calycanthine molecule. By combining fragment (I) with quinoline it is possible to arrive at (II). The quinoline is shown attached to the



(I)



(II)

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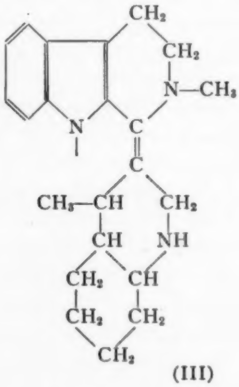
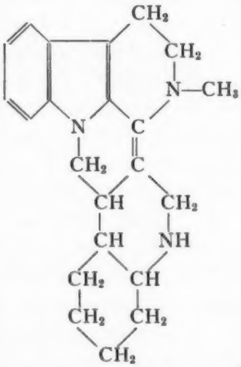
[2] *Chemist, National Research Laboratories, Ottawa.*

carboline fragment at carbon-atom-2 because that position permits a better understanding of the results of the degradation of the alkaloid with selenium, and the 3- position of the quinoline nucleus is chosen in anticipation of some results which follow.

As already reported (8) the selenium degradation yielded norharman and a base for which we are pleased to retain the designation *calycanine* suggested by Barger *et al.* (1). In a more thorough study of this degradation it has now been found that there is also obtained β-methyl-indole, β-ethyl-indole, and lepidine. The source of the norharman can be regarded as the carboline-half of the molecule since the pyridine ring of the latter cannot become aromatic unless it loses its N-methyl group. Likewise, β-methyl- and β-ethyl-indole are undoubtedly formed from the same fragment. However, the isolation of lepidine makes it possible to extend the partial structural formula (II) to (III).



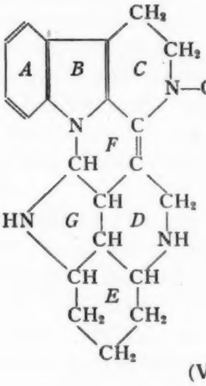
(III)



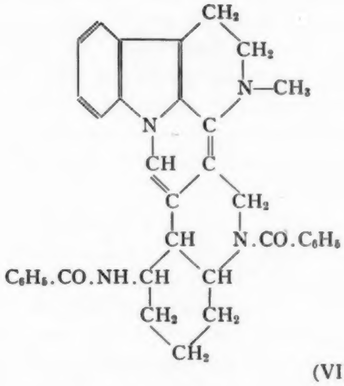
(IV)

Since Barger and his co-workers (1) report the absence of a C-methyl group in calycanthine, it is further possible to link the methyl group of lepidine to the indole nitrogen as in (IV). A search for a 1-methyl-indole derivative in the products of the selenium degradation of calycanthine has proved fruitless. In view of the ease with which a methyl group so situated is eliminated in pyrolytic reactions (5, p. 181; 6), and in view of its presence in the lepidine, it is not thought that the failure to find it in the indole moiety is sufficient evidence that this nitrogen is not substituted in calycanthine. Furthermore, the absence of a C-methyl group in the alkaloid is proof that the methyl group of lepidine must be produced during the degradation. In addition, the fact that, depending on conditions, quinoline or lepidine are obtained as degradation products militates against the occurrence of the methyl group of lepidine as such in calycanthine.

When calycanthine is heated in a stream of nitrogen with palladium, ammonia is obtained. To account for the ease with which ammonia is thus liberated, the fourth nitrogen atom of calycanthine can be included in a reduced pyrrole ring *G* as in (V), thus completing the structural formula of the alkaloid for which the empirical formula $C_{22}H_{26}N_4$ is here accepted rather than the double formula of Gordin.



(V)



(VI)

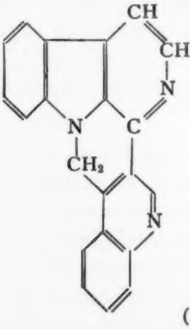
Additional support for the introduction of the fourth N-atom as in ring *G* is to be found in the fact that the N-C bond from rings *B* to *G* is severed easily in the benzoylation experiments (7). As formulated, the nitrogen of ring *G* would probably not be basic to the extent that calycanthine should form a trihydro-halide. It is assumed that it is this nitrogen atom which is so readily eliminated (as methyl-amine) in methylation experiments (1, 4, 8). The introduction of oxygen during these experiments is then readily explicable on the basis of simple hydrolysis. The possibility that the readily eliminated methyl-amine might come from the N-methyl group of ring *C* should not be overlooked, although it is considered that the above-mentioned production of ammonia does not favour such a view.

In the hope of obtaining a clue as to the fate of rings *D* and *E*, a further study of the benzoylation experiments was made. Ultimately, besides benzoyl-N-methyl-tryptamine, an amorphous acid was obtained which contained nitrogen and one or more benzoyl groups. This acid yielded an amphoteric substance when debenzoylated, and quinoline when heated with selenium. The acid is, therefore, probably a largely hydrogenated (5)?-amino-quinoline-3 : 4-dicarboxylic acid in which the nitrogen atoms are lactamised or benzoylated. The nitrogen content supports this assumption. Benzoyl-N-methyl-tryptamine, when similarly treated with selenium, yielded no quinoline.

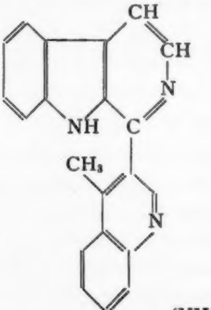
In one benzoylation experiment already reported by one of us (7) a basic substance was obtained, m.p. 235° C. The analytical figures are in excellent agreement with the empirical formula $C_{36}H_{34}O_2N_4$ which, on the basis of structure (V), for calycanthine can be expanded to structure (VI). The steps involved are,—severing of the N-C bond of the five-membered ring *G* by hydrolysis, benzoylation of the nitrogen, elimination of water with the formation of a double bond, as well as benzoylation of the secondary nitrogen of ring *D*.

Calycanthine has not been found to be reducible and is recovered unchanged after treatment with sodium in butyl alcohol. Most double bonds present must presumably form part of aromatic rings. The fact that the double bond of ring *F* in structure (V) was not reduced by this treatment may be attributed to steric hindrance. As already reported (8) calycanthine, when oxidized by Gadamer's method, loses two hydrogen atoms which can be readily added again by reduction. Since the product thus obtained is identical with calycanthine, no stereoisomeric changes appear to be involved, and the two hydrogen atoms concerned are probably removed from the two methylene groups of ring *C*.

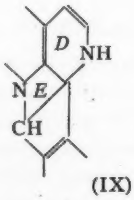
As mentioned above, the degradation of calycanthine with selenium yielded a base which Barger and his co-workers (1) have named calycanine. Although we had temporarily (8) suggested the empirical formula $C_{16}H_{10}N_2$ for this base, further examination and analysis of a purer sample shows it to be in good agreement also with $C_{21}H_{13}N_3$ (mol. wt., 307) or $C_{21}H_{15}N_3$ (mol. wt., 309). A determination of the molecular weight gave an average figure of 614 which is twice the required weight, but this might be due to association. Furthermore, calycanine fails to give a colouration with Ehrlich's reagent, indicating that no α- or β- position in the indole nucleus is unsubstituted. On the basis of formula (V) for calycanthine, this degradation product would be expected to be one formed by loss of ammonia and hydrogen. In order that ring *C* become aromatic the methyl group must be eliminated from the nitrogen, and thus we arrive at either formula (VII) or (VIII) for calycanine.



(VII)



(VIII)

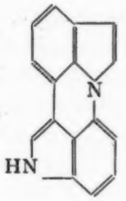


(IX)

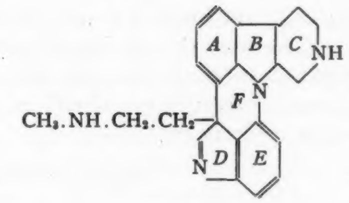
Formula $C_{21}H_{15}N_3$ (VIII) is consonant with the presence of one active hydrogen (1) and contains the methyl group of lepidine, although the tautomeric form of (VII), involving a para link and the wandering of a hydrogen as in (IX), would account for an active hydrogen.

We believe that formula (V) for calycanthine is a reasonable expression for the gross constitution of the alkaloid, although further experimental evidence is necessary to elucidate the detailed structure.

Barger and his co-workers (1) have proposed constitutional formulae for calycanine (X) and calycanthine (XI) which apparently are based upon preconceived notions of biogenesis



(X)



(XI)

and, we believe, a misrepresentation of experimental facts. It is not deemed essential to discuss their formulae in detail, but some errors and inconsistencies deserve mention. The discussion of the biogenesis from two molecules of tryptophane is introduced in their paper by way of the erroneous statement that calycanthine has been isolated from a plant of the family, Compositae, but *Meratia praecox* in common with the genus Calycanthus belongs to the Calycanthaceae family.

We have isolated calycanthine from two other species of *Calycanthus*, namely, *C. occidentalis* Hook. et Arn. which yielded 0.8% of pure alkaloid and from *C. glaucus* Willd. (*C. fertilis* Walt.). For the latter we are indebted to Dr. Alfred Rehder of the Arnold Arboretum, Jamaica Plains, Mass. In both cases the alkaloid was isolated by the procedure used for *C. floridus*, thus it is not considered essential to give experimental details.

In the discussion of their formula (X) for calycanine, Barger *et al.* have overlooked the fact that calycanine does not give Ehrlich's reaction. This negative result indicates that the indole nucleus of calycanine must be substituted in both the α- and β-positions, whereas in formula (X) two α- and one β-carbon atoms are unsubstituted. Furthermore, calycanine is basic and it is highly probable that the compound represented by structure (X) would be neutral.

In support of their formula (XI), the above-mentioned authors (1, p. 513) state that in the course of the reaction yielding N-methyl-tryptamine the bond between the quaternary atom and ring *A* is ruptured (along with that between *B* and *E*), and that the fission is brought about by soda-lime, but not by quick-lime. They conclude that the fission is evidently a hydrolysis rather than a pyrolysis. Yet, in the same paper (1, p. 517) they mention that 30 mg. of N-methyl-tryptamine is obtained per gram of alkaloid when calycanthine is heated with quick-lime at 305° C. for four hours.

The same authors have isolated 2-phenyl-indole as a product of the degradation of benzoyl-calycanthine but have failed to obtain it from calycanthine itself. We also have failed to detect any trace of phenyl-indole among the degradation products of calycanthine and are of the opinion that the phenyl group comes from the introduced benzoyl radical. It is difficult to see how 1-, 2- or 3-phenyl-indole could be derived from the degradation of a structure such as (XI).

Finally, a compound such as (XI) should react with phenyl-isocyanate giving rise to a neutral di-phenylcarbamyl derivative. Calycanthine, as already reported (8), does form a di-phenylcarbamyl derivative, but the latter is basic. In our formula (V) the two secondary nitrogens of rings *D* and *G* would be expected to condense with phenyl-isocyanate and the resulting bisphenylcarbamyl derivative would still be a base owing to the basic nature of the tertiary nitrogen in ring *C*.

Experimental

Degradation of Calycanthine with Selenium

Anhydrous calycanthine[*] (2 gm.) was heated in a metal bath with selenium (2 gm.) in a stream of nitrogen. The reaction was carried out in a distillation flask connected through a condenser to a receiver. The temperature was gradually brought up to 300° C. and maintained for one-half hour. The flask and the melt, which had set to a hard resin, were crushed together to a powder in a mortar, and the distillate kept separate. Four more runs, each with 2 gm. of calycanthine, were made and the respective products combined.

Isolation of Skatole

The combined distillates from the five runs were dissolved in ether and the ethereal solution was washed with dilute hydrochloric acid, dilute sodium hydroxide, and water. The ethereal solution yielded a neutral brown oil which was distilled. A first fraction, b.p. 115° C./1–1.5 mm., was separated which formed a red picrate, m.p. 166°[†]. When mixed with skatole picrate (m.p. 178°) it melted at 172°. After recrystallization from absolute ether (1 cc.) it melted at 171° and, in admixture with skatole picrate, at 173.5°.

Isolation of β-Ethylindole

The distillation of the neutral product yielded a second fraction, consisting of a colourless oil, b.p. 125 to 130°/1 mm., wt. 0.262 gm. This was treated with picric acid (0.417 gm.) in ether solution containing a few drops of methanol and the picrate precipitated by the addition of petroleum ether. It was recrystallized once from benzene and once from absolute ether, from which it separated as red needles, m.p. 121° C. The indole was regenerated by shaking an ethereal solution of the picrate repeatedly with aqueous potassium hydroxide and the neutral oil redistilled; b.p. 110°/5 mm. The distillate crystallized immediately in colourless flakes, m.p. 39°, either before or after admixture with β-ethyl-indole. It was reconverted into the picrate, m.p. 120.5°. Admixture with β-ethyl-indole picrate (m.p. 120.5°) failed to depress the melting point.

In this connection, it may be noted here that β-ethyl-indole (m.p. 39 to 40° C.) undergoes on long standing a transformation into a compound, m.p. 81 to 82°, which depresses strongly the melting point of β-ethyl-indole when mixed with it. The transformation product forms a picrate, m.p. 127°, which in admixture with β-ethyl-indole picrate melts at 108°.

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[*] *In one recrystallization from chloroform-methanol, calycanthine was obtained in needles melting at 214° C. An attempt to recrystallize this yielded the base melting at 245° C. The low-melting form obviously consisted of Gordin's iso-calycanthine (3).*

[†] *All melting points are corrected.*

Isolation of Lepidine

The hydrochloric acid extract obtained in the course of isolating the indoles from the ether solution of the distillate was basified with strong caustic, which precipitated a granular base. The mixture was shaken with several portions of ether and a small quantity of insoluble product filtered and washed (basic fraction *A*). The ethereal solution yielded a mixture of crystals and oil. It was digested with cold ether which dissolved only the oil. The crystals were combined with basic fraction *A* and the ethereal solution was extracted with dilute hydrochloric acid. The acid solution was filtered through charcoal, basified, and extracted with ether. The mixture of bases obtained from the ether extract was treated with picric acid in methanolic solution. The crystalline picrate (*B*) was filtered off, and the filtrate evaporated to a very small volume, from which a second crystalline picrate was deposited. This, after repeated recrystallization from methanol, melted at 217°. In admixture with lepidine picrate (m.p. 218°) it melted at 217.5°.

The melts obtained from the dehydrogenation with selenium were ground and extracted with ether in Soxhlets. The ethereal extract was washed with dilute hydrochloric acid. The neutral fraction yielded some β-ethyl-indole. The acid solution was basified with strong potassium hydroxide and the precipitated bases were shaken with ether. Some insoluble product was filtered, washed with ether, and combined with basic fraction *A*. The ether solution was then distilled and the residue digested with some cold ether, the insoluble portion being combined with basic fraction *A*. The bases recovered from the ether solution were distilled *in vacuo* and separated into three fractions: I, b.p. 80 to 85°/1 mm.; II, b.p. 100 to 130°/1 mm.; III, b.p. 170 to 180°/1 mm. Fractions I and II when treated with picric acid yielded lepidine picrate, m.p. 217° before and after admixture with an authentic specimen.

Isolation of Norharman

Fraction III crystallized on standing. It was recrystallized from ether from which it separated as small prismatic needles, m.p. 187°. It was converted to a picrate, which melted at 261°, and the free base as well as the picrate did not depress the melting points of 3-carboline (norharman) or its picrate*.

The mother liquor from norharman picrate was combined with the crystalline picrate *B* and decomposed. The bases were again fractionated *in vacuo* and the high-boiling fraction (b.p. 170 to 180°/1 mm.) was converted to the picrate. After a series of fractional crystallizations a picrate was finally obtained which melted at 264 to 265°. The melting point was depressed after admixture with the picrates of norharman, of 1-methyl-3-carboline, and of harman. This base, the solution of which in hydrochloric acid fluoresces, seems to be a carboline, but may not be quite pure.

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* *This isolation and characterization has been detailed already (8), but norharman was then described as 4-carboline. We prefer the more recent nomenclature and refer to it as 3-carboline.*

Isolation of Calycanine

All the basic fractions *A* were combined, dissolved in dilute hydrochloric acid, filtered through charcoal, and precipitated by basifying the solution with strong potassium hydroxide. The base was filtered, washed with water, dried, and dissolved in boiling chloroform. Boiling ethyl alcohol was added to the solution (charcoal) which was then evaporated to a small volume and diluted with boiling ethyl alcohol. The base separated as soft white needles, m.p. 308°. It was sublimed at 210° C./0.001 mm. and recrystallized again from chloroform-ethyl alcohol, m.p. 310°. Calcd. for $C_{21}H_{13}N_3$: C, 82.09; H, 4.24; N, 13.68%.; mol. wt., 307. Found: C, 82.43; 82.70; H, 4.75, 4.54; N, 12.60, 12.68%; NCH_3, negative; mol. wt., 584, 626, 642, 604 (Rast). Calcd. for $C_{21}H_{15}N_3$: C, 81.23; H, 4.85; N, 13.59%; mol. wt., 309.

Treatment of Calycanthine with Palladium

Calycanthine (2 gm.) was mixed with Pd-charcoal catalyst (2 gm.) in a distillation flask carrying a capillary tube, and connected to a condenser reaching into a suction flask. The flask carried a tube dipping into dilute hydrochloric acid. A stream of nitrogen was kept sweeping into the flask through the capillary while the experiment lasted. The mixture was heated to 260° to melt the calycanthine and then maintained at 200 to 210° for five hours. The hydrochloric acid solution in which the gases had been washed was evaporated to dryness, the crystalline residue dissolved in methanol, and a 5% solution of platinic acid (3 cc.) poured into the solution. A brown, amorphous precipitate which separated was filtered and washed with methanol, and the combined filtrate and washings evaporated to about 2 cc., filtered, and evaporated to dryness. The residue was redissolved in a little boiling methanol, and allowed to crystallize on cooling. The lemon-yellow crystals decomposed at 300°, giving off what appeared to be ammonium chloride. Calcd. for $(NH_3)_2 . H_2 PtCl_6$: Pt, 43.9%. Found: Pt, 42.9%.

The reaction flask contained some unchanged calycanthine which was mixed with a base the solutions of which in dilute acid were strongly fluorescent. It has not yet been possible to isolate this base in a pure condition.

Benzoylation of Calycanthine

As already reported (7), calycanthine when benzoylated and subsequently oxidized in acetone with potassium permanganate yields benzoyl-N-methyltryptamine. It has now been found that when the manganese dioxide sludge, after filtration and washing with acetone, is digested with boiling water, and filtered, the filtrate when acidified yields a mixture of benzoic acid and another acid which is only sparingly soluble in ether. Use is made of this property to remove the benzoic acid. The acid, which melts at 170 to 174°, could not be crystallized. It contains nitrogen and gives a positive reaction with Ehrlich's reagent on prolonged boiling. Calcd. for $C_{18}H_{18}O_4N_2$: N, 8.59%. Calcd. for $C_{18}H_{20}O_3N_2$: N, 8.77%. Found: N, 9.26, 9.62%.

In order to determine whether this nitrogenous acid contained combined benzoic acid, some was dissolved in methanol (5 cc.) and refluxed 16 hr. with concentrated hydrochloric acid (10 cc.). Water (200 cc.) was added and the cooled liquor extracted with six portions of ether. The combined ether extracts yielded a crystalline residue which was recrystallized from water, m.p. 121.5°. In admixture with an authentic specimen of benzoic acid it melted at 122 to 123°. The aqueous hydrolysis liquor, which had been extracted with ether, was evaporated to a small volume, whereupon a crystalline substance separated which is being further studied.

Isolation of Quinoline

Some of the benzoyl-nitrogenous acid (0.4 gm.) was heated with selenium (5 gm.) in a metal bath, while a stream of nitrogen was kept sweeping through the flask. The temperature was gradually raised to 300° and maintained for one-half hour. The flask and melt were crushed in a mortar, suspended in very dilute potassium hydroxide, and steam distilled. The distillate (150 cc.) was repeatedly extracted with ether, and the combined extracts were washed with two portions of dilute hydrochloric acid. The acid solution was basified with strong potassium hydroxide and extracted with ether. The ether extract yielded a small quantity of oil which was treated with a methanolic solution of picric acid. The crystalline picrate which separated was recrystallized several times from methanol. It melted at 203.5° C. Admixed with an authentic specimen of quinoline picrate, m.p. 204°, the mixture melted at 204°. Some benzoyl-N-methyl-tryptamine (0.5 gm.), when similarly treated with selenium (5 gm.), did not yield quinoline in quantity sufficient for identification.

In an experiment already reported by one of us (7) benzoylated calycanthine was heated for several hours on the steam bath with alcoholic potassium hydroxide; it yielded among neutral and acidic fractions a moderate quantity of a basic substance, which is soluble in aqueous citric acid and crystallizes from alcohol in stout plates, m.p. 235°. It does not give a colour with Ehrlich's reagent. Calcd. for $C_{36}H_{34}O_2N_4$: C, 77.98; H, 6.14; N, 10.11%; mol. wt., 554. Found: C, 77.66, 77.84; H, 6.16, 6.07; N, 10.10, 10.07%; mol. wt., 517, 496.

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MICROCHEMICAL TECHNIQUE

III. SEMI-MICRO PREPARATION AND PURIFICATION OF ORGANIC SUBSTANCES[1]

By George F. Wright[2]

Abstract

Several devices convenient in semi-micro preparation and purification of organic substances are described. A modified side-arm test tube is suggested as of general utility in microchemical laboratory technique.

The introduction of quantitative microanalytical methods has facilitated organic chemical research both because of the small amounts required for analysis and because of the time saved in carrying out the semi-micro or micro reactions required for their preparation. The convenience and rapidity of the small-scale procedure has, however, been offset by the fault that quantitative recovery of the products is often difficult, and the significance of the experiment is decidedly lessened thereby. While much of the general microchemical technique is actually older (3) than the quantitative methods of Pregl, the continued rapid advances in the latter art require constant improvement in preparative and purification methods. It seems worth while to report at this time several devices that the author and his co-workers have found useful in manipulation of 1 to 100 mg. of organic substances.

Solubility Tests

A great deal of preliminary information pertaining to solubility, crystallizability, and reactivity may be obtained with the microscope slide using 100 γ of substance not sensitive to moisture or air. If care is taken, this amount suffices, first for successive solubility tests with organic solvents and water, and subsequently for solubilities in acid and alkali. In the solubility and crystallizability tests each solvent is evaporated before the next is added. To effect this evaporation conveniently the slide is placed on the flat top of a large cork borer, mounted vertically, and heated by a microflame (*A*, Fig. 1). This device enables one to heat the slide without undue spreading of the solution over the surface. The test reagents must be applied in small quantities. Suitable containers may be made from glass tubing drawn (Fig. 2) out at one end to a fine thick-walled bent capillary which is at first sealed. The tube is then constricted at the other end, filled with the reagent and then sealed off. The pointed end which remains after sealing is of advantage if the reagent tubes are kept in the ordinary test tube rack. An alternative method of filling, satisfactory with reagents boiling above 50° C., is to seal the lower end of the tube under vacuum and then to break the tip in a beaker of the substance. These tubes have the advantage of delivering clean reagents.

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Contribution from the Chemical Laboratory, University of Toronto, Toronto, Canada.

[2] *Assistant Professor of Chemistry, University of Toronto.*

Leakage through the capillary is no greater than through the ordinary glass stopper; however, sensitive or little-used reagents may be preserved by sealing the tip of the capillary with a microflame. The reagent container is manipulated by tipping the tube while it is held lightly in the hand. The body heat imparted by a firmer grasp will then deliver a drop to the appropriate position on the slide. When low boiling solvents, such as ether, are used there is danger that the high vapour pressure forces out more than one drop of the solvent; this difficulty can be obviated by tipping the cold tube so that, when it is tipped back to vertical, one drop is held in the bent juncture between the capillary and the body of the reagent tube. Upon warming the container with the hand this drop may then be forced out on to the slide. It should be pointed out that this method of reagent delivery supplements rather than replaces the use of the micropipette.

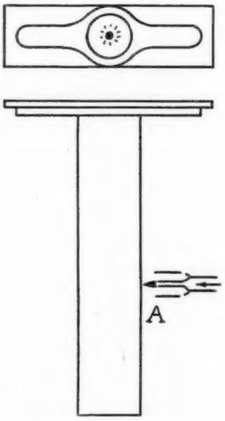

FIG. 1

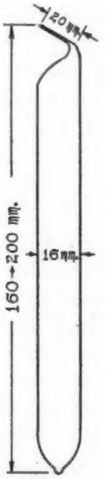

FIG. 2

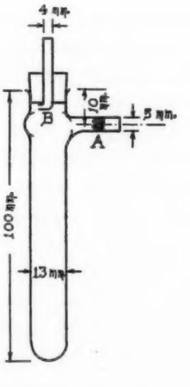

FIG. 3

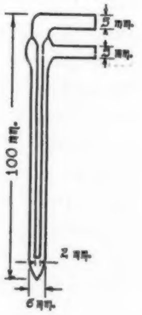

FIG. 4

The determination of solubilities on the microscope slide is necessarily less exact than that outlined by Foulke and Schneider (4) using microcapillaries, but is much more rapid. As a guide toward choice of crystallizing solvent it is more informative; the favourable solvent is obviously that from which the crystalline material separates at the periphery of the spot leaving the impurity at the centre. Magnification, either with the hand glass or the low power microscope, is convenient in ascertaining this solubility difference. Caution must be exercised regarding results with hygroscopic solvents—atmospheric moisture may condense in the evaporating solution and thereby change the characteristic solubility.

The Side-arm Test Tube

This well known piece of apparatus has been modified as shown in Fig. 3 by blowing an enlargement about 5 to 10 mm. below the tip of the flanged 10 by 75, 13 by 100, or 15 by 125 mm. Pyrex test tube and attaching a side-arm to the bulge in such a manner that a trough leads from the tube to the lower side of the arm. The purpose of the alteration is to eliminate contamination of the stopper when liquid is decanted through the side-arm. The tube is of general utility in micromanipulation. Thus, it is used in this laboratory as a reaction vessel, dropping funnel, crystallizing vessel, etc.

For use in crystallization, a plug of long-fibered cotton wool or fibrous glass (Corning) is tamped into the side-arm as illustrated at *A*, Fig. 3, and a cork (previously boiled or Soxhlet-extracted in ether or acetone to remove soluble constituents) is inserted into the mouth of the tube after it has been equipped with a glass tube pulled off sharply to one side *(B)*. A 3- to 4-ft. length of small-bored rubber tubing is attached to the straight end of the tube *B*. The sample and solvent are placed in the side-arm tube, which is then inclined at an angle with the side-arm up, and is heated in a suitable bath (conveniently over a hot plate) until the solvent is refluxing smoothly. Occasional gentle puffs of air are blown in by mouth through the rubber tubing in order that the solvent will not contaminate tube *B*, but tends, instead, to moisten plug *A*. When the sample is properly dissolved, the hot solution is decanted through the side-arm into a second, previously warmed, test tube. Just enough air pressure is applied by mouth to keep the trough of the crystallizing tube from over-filling, but care should be taken not actually to clear the plug of liquid. A few drops of excess solvent suffices to clean the tube of the soluble constituent. Any undissolved residue remains in the crystallizing tube, from which it may be further extracted with a more suitable solvent. It is evident that this method of crystallization obviates the losses incurred by filtering through a funnel. Because the tube is of uniform bore it is a simple matter to ascertain, by marking the tube with a wax pencil, the minimum amount of solvent or solvents required to effect solution; this makes it possible to record and reproduce the crystallizing conditions. Its disadvantage, namely, loss of solvent, which must be avoided when crystallizing from mixed solvents, may be remedied in the case of slow, difficult solution by replacing the blowing tube *B* with a cold finger (Fig. 4) during the dissolving process. This cold finger, useful when the side-arm test tube is employed as a refluxing reaction flask, is constructed from tubing of 6 mm. outside diameter by blowing an enlargement on a closed tube of this diameter and inserting into it by ring seal a tube of 2 to 3 mm. diameter, the side-arm being subsequently attached.

The use of the side-arm test tube as a reaction vessel and dropping funnel may be illustrated in the characterization of organic halides by means of their Grignard reagents (5). The halide in ether solution is placed in a side-arm test tube equipped with a boiled, dried cork stopper on the arm and a stoppered inlet tube that introduces a slow stream of dry nitrogen. This tube is attached to a second dry side-arm tube which contains the magnesium (Fig. 5). When the system is flushed with nitrogen, the side-arm of the second tube is connected by rubber tubing to the nitrogen manifold; this creates a system isolated under nitrogen atmosphere. A plug of cotton at the nitrogen exit provides a slight positive pressure. The halide is introduced by turning the first tube around the axis of its side-arm so as to effect a dropwise addition to the magnesium, the second tube being maintained at an angle of approximately 45° to insure smooth boiling. Reflux is obtained by cooling the upper part of the tube with a compressed air jet. When addition is complete and reflux has subsided, the prepared reagent is decanted dropwise through a fibrous glass plug (to free it of unreacted magnesium) into a third side-arm test tube, swept out with nitrogen from the manifold and containing the solution of characterizing reagent.

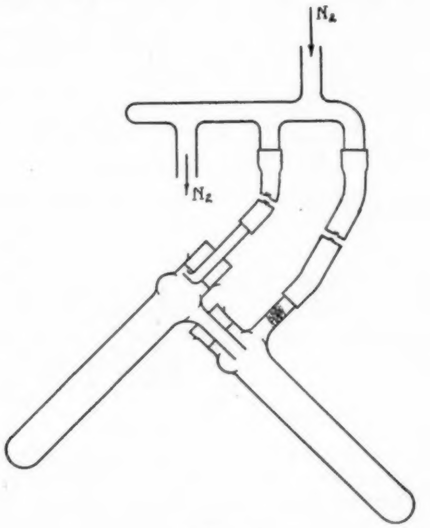

FIG. 5

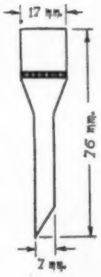

FIG. 6

The Suction Funnel

It is a rule of micromanipulation that, whenever possible, a solution or suspension should not be poured over the lip of a vessel. The sample mentioned above should therefore have been allowed to crystallize in a second side-arm test tube, from which it may be removed by decanting through the arm. There are a number of small-sized suction funnels (Büchner type) available on the market that are suitable to receive this crystalline suspension. The type (Fig. 6) used in this laboratory differs from the conventional porcelain or glass types in that it is made of Pyrex glass with a sealed in porcelain disc. The funnel has been built to accommodate the bevelled, glazed porcelain plates of sizes 22 mm., 14 mm., and 7 mm. diameter. Modifications of this construction have been used for low temperature crystallization apparatus (2) and for continuous ether extractors, in lieu of the sintered glass plates.

The porcelain glass seal is made, especially in the larger sizes, by "tacking" the disc in place at one spot while holding the previously constructed glass part vertically, and then heating a length about ½ inch on either side of the seal with a bunsen burner while an oxygen-gas flame about the size of a darning needle is focused directly on the juncture of porcelain and glass. Such a flame is desirable in order to get a complete wetting or fusion of glass into porcelain, so as to avoid spaces which may become fouled with use. This small sharp flame may be obtained by connecting the two inlet tubes of an ordinary nasal atomizer with gas and oxygen supplies. The supply of oxygen is easily adjusted by means of a needle valve (obtained in the writer's case from an old suction-type automobile windshield wiper) soldered on to the screw top of the atomizer so that it opens directly into the glass bowl. The bowl is then filled with lead shot and plastic cement to furnish stability to this improvised torch, as well as to fill up the dead air space, since the tip will work satisfactorily only with oxygen and not with air. The torch is illustrated in Fig. 7. Its small but very hot flame is useful in such operations as the construction of the ring-seal shown in Fig. 4.

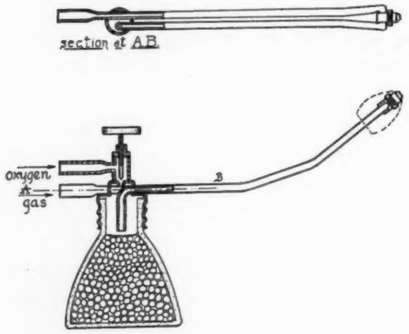

FIG. 7

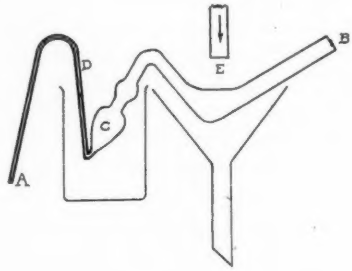

FIG. 8

When the porcelain-glass seal is completed it requires careful annealing because the disc holds its heat so much longer than the glass around it. To test the efficiency of this annealing process, the completed funnel is heated to 100° C. and then chilled in cold water. A properly annealed funnel does not break under such treatment; those that survive the chilling test have been in use for over five years.(*)

The Microdistillation Flask

This method of distillation is an adaption of that developed by Bendetti-Pichler and Schneider (1). The apparatus is constructed from glass tubing

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* *These funnels may be obtained from the firm, Eck and Krebs, 131 West 24th Street, New York, N.Y.*

3 to 7 mm. outside diameter and is bent into the shape illustrated in Fig. 8. The liquid to be distilled is introduced into the pot *C* by dipping the capillary *A* into the liquid and drawing it up by partial vacuum applied at *B*. In the case that the liquid to be distilled is dissolved in a solvent such as ether or benzene, this solvent may be "flashed off" by surrounding the distilling pot *A* with a heated beaker of water or wax, the suction being carefully controlled, conveniently by using an evacuated desiccator as a vacuum chamber. When the substance to be distilled is transferred entirely into the distilling pot, the capillary is sealed off at *D*, with the same mild suction; full vacuum is applied and then released so that the capillary becomes almost filled. A tuft of long-fibered cotton wool is inserted into *B* in such a way that the rubber tubing leading to the vacuum pump holds it so that it cannot be carried along by the gas stream. This is accomplished simply by allowing a few strands of the plug to extend over the outside of tube *B*, though not far enough to cause vacuum leakage at this point. A means of cooling, either of water flowing over the receiver *E* into a funnel, or of dry ice packed around *E* in a shallow dish, is employed. The vacuum required for distillation is then applied and the temperature of the stirred bath is slowly raised until the almost filled capillary empties itself; this represents the initial boiling point of the substance. When fractionation is to be effected, the vacuum is slowly released through the cotton plug, which is then withdrawn and the fraction removed by means of a capillary pipette. The capillary at *D* is broken, resealed under slight suction, and the distillation continued as before. By extending (to 5 cm.) and indenting the column of such an apparatus built from tubing 3 mm. outside diameter, Mr. E. Y. Spencer, of this laboratory, has successfully fractionated 0.1 ml. of liquid into two substances boiling at about 80 and 90° C. under 15 mm. pressure.

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THE REMOVAL OF FLUORINE FROM ALBERTA WATERS[1]

By Osman James Walker[2], Gordon Roy Finlay[3], and Walter Edgar Harris[4]

Abstract

The fluorine removing capacities of a large number of materials have been investigated. Many of these have been found to have little or no effect on the fluorine content of the water, while others have lowered the fluorine content but not enough to prevent mottling of the teeth.

Two materials, specially prepared aluminium oxide and freshly precipitated aluminium phosphate, have been examined and have been found to be equal to, or better than, other materials which have been proposed previously. These two have been compared with activated alumina, tricalcium phosphate, and magnesium oxide, materials reported upon by other writers.

Experiments on these have been carried out using the filtration technique and the stirring and standing technique with good results.

A commercial defluorite unit, containing tricalcium phosphate, supplied by the National Aluminate Corporation, has been tried out at three points in Alberta and found to have a high fluorine removing capacity.

In 1931 Smith and her co-workers (10, 11) and Churchill (4) established the fact that the mottling of teeth is caused by the presence of fluorine in the water supply. It has since been established that the effect on the teeth takes place between the ages of six months and twelve years (5), that is, during the period when the teeth are calcifying within the gums, and that amounts of fluorine (8) over 1 p.p.m.* may lead to mottled enamel. A great deal of thought has been given to the prevention of this tooth defect, as experience has shown that if the teeth are once attacked, the mottling cannot be removed by any method which is now available. It is therefore necessary to prevent mottling in the first place by providing children with a water supply that contains less than 1 p.p.m. of fluorine.

There are two avenues open to residents in many endemic districts. The first method is to switch over from a water high in fluorine to a water low in fluorine (5). This has been done very successfully in a number of localities in the United States. Surveys carried out in the Province of Alberta (13, 14) show that there are some districts where such a change is possible, as the water from shallow wells and streams is generally low in fluorine content even though water from the deep wells is high in this element. In some of these cases it may involve the transportation of water a considerable distance, especially in the dry seasons when shallow wells tend to dry up. In many districts such a step is impossible, as there may not be available an adequate supply of low fluorine, potable water.

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[1] *Manuscript received June 9, 1939.*
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* *p.p.m. is an abbreviation for parts per million parts of water.*

The second method of preventing mottled teeth is to start with a water containing over 1 p.p.m. of fluorine and, by means of some material, lower the fluorine content to less than 1 p.p.m. Since the amount of fluoride involved is so small, precipitation methods are not feasible, as the least soluble fluorides known are soluble to a greater extent than 1 p.p.m. The formation of complex ions containing fluorine may offer some possibilities especially if the resulting compound has a low solubility in water. It may be possible to remove the fluorine from water by a system of anion exchange resembling the base exchange softening of water by zeolites. Of greater importance is probably the removal of fluorides from the water by selective adsorption on the surface of a solid. A combination of one or more of these processes may be the mechanism by which satisfactory removal of fluorine takes place. Since the amount removed is very small it is rather difficult to tell whether one has complex ion formation, anion exchange, or selective adsorption on the surface of a solid.

In order that a material may be used as a fluorine remover it should have a low solubility in water. It should be of proper grain size, say 20 to 40 mesh, so that it has a sufficiently large surface and yet will not remain in the treated water as suspended matter. It should not change the potability of the water. It should be comparatively cheap so that it will be available for country users, many of whom live in districts where crop failures are frequent.

During the last three years, investigators have been busy trying to find methods of removing fluorine from natural waters. The National Aluminate Corporation first used activated alumina (7, 12) for this purpose, but later transferred their attentions to tricalcium phosphate (1, 2) and are now manufacturing commercial units containing this material for fluorine removal. Smith and Smith (9) have recommended the use of crushed bone, while Elvove (6) has suggested magnesium oxide for this purpose. The Permutit Company Limited (3) has patented the use of various dried metallic oxide gels.

Two different techniques have been used in the treatment of waters. The one which has been used by most of the investigators is to pass the water through a column of the remover at such a rate that the fluorine content is lowered below the threshold level of 1 p.p.m. The other scheme is to add a small amount of the solid to a vessel of water, stir, and allow to stand in contact for some time, usually overnight, and then draw off the clear liquid. Fresh water can then be added and the operation repeated until the solid has lost its fluorine removing properties. These two methods have their advantages. In the percolation or filtration method a large amount of the remover may be used at one time, so that large volumes of water may be treated and little supervision is required. When the material has lost its effectiveness it is removed and either discarded or sent to the laboratory to be regenerated. This is the ideal method to be used when the water supply is piped into the home. The other method, sometimes called the spoon and bucket method, can be used most conveniently when the water is taken from the well to the home in barrels or buckets. Only the water for drinking or for cooking need be treated, and, since the amount of remover is very small, it may be discarded after having been used once or twice.

The investigation of fluorine removers by the present authors was begun in 1937 prior to the appearance of many of the papers which have been mentioned. Both procedures have been used. A large number of materials have been examined, many of which have little or no fluorine removing power. Others have been found to remove fluorine but not enough to lower the fluorine content below 1 p.p.m. Several have been found to be excellent for the purpose in addition to those reported by other investigators. It was also found that the fluorine removing capacity depended on whether the material was freshly prepared or had been taken from stock bottles, and also on the method of drying and the temperature of drying.

The water used in most of the investigations was that delivered from the taps in Edmonton, the source of the supply being the North Saskatchewan River. To this was added sufficient sodium fluoride to bring the fluorine concentration up to a definite amount, usually 4 p.p.m., but other concentrations were used in some experiments. The composition of Edmonton city water (in p.p.m.) in September, 1937, was as follows:

Total dissolved solids at 105° C.	200
$SO_4^=$	30
Cl^-	4
Bicarbonate alkalinity (temporary hardness)	110
Carbonate alkalinity	0
Total hardness (soap method)	152
F^-	0.2
pH	8.2

During the winter months the mineral content may be as much as 70% higher than the values shown here. A number of tests have also been conducted on high fluorine Alberta waters, but these have been limited in number owing to difficulties in transporting sufficient quantities to the University laboratories.

The following materials were found to have no fluorine removing properties: titanium oxide, titanium hydroxide, zinc oxide, copper hydroxide, manganese dioxide, hydrated manganese dioxide, various silica gels, bentonite, zirconium silicate, beryl, ilmenite, a limy subsoil, copper sulphide, flowers of sulphur, calcium oxalate, calcium oleate, trimagnesium ortho-phosphate, barium carbonate, barium sulphate, magnesium oxychloride, chromium borate, Portland cement, permutite, boracite, and limestone.

Materials with a low capacity for the removal of fluorine were basic ferric carbonate, magnesium ammonium phosphate, aluminium oxalate, powdered aluminium, and various hydrated ferric oxides precipitated on asbestos and dehydrated at different temperatures. In no test with these materials was the fluorine content of the water lowered to a value less than 1 p.p.m. However, the aluminium oxalate, which was ineffective in earlier runs, showed a fair capacity when further amounts of water were treated.

Removal of fluorides was observed with magnesium oxide as used by Elvove (6), and with activated alumina (8) and tricalcium phosphate (12) supplied by the National Aluminate Corporation. In addition, results were obtained with tricalcium phosphate supplied from various sources, hydrated aluminium oxide given different heat treatments, a mixture of aluminium oxide and calcium carbonate, and freshly prepared aluminium phosphate. The best results were obtained using aluminium oxide dried at 81° C., and aluminium phosphate.

In the first series of experiments the materials used were different forms of alumina which had been dried under different conditions. The description of these fluorine removers is as follows. For Run 1, the aluminium oxide had been dried at red heat; for Run 2 the aluminium oxide had been dried at 81° C. for four hours; for Run 3, the drying had been carried out at room temperature; while for Run 4, aluminium hydroxide had been precipitated on shredded filter paper, air dried for two days, and then heated for 12 hr. at 190° C. For Run 5, activated alumina (Defluorite *A*) supplied by the National Aluminate Corporation, was used.

In these experiments the materials were placed to a height of about 50 cm. in tubes about 2.0 cm. in diameter. The water used contained 8 p.p.m. of fluorine. Gravity flow was used in all runs.

There was a considerable variation in specific gravity of these materials and thus a considerable variation in the weights used. The amounts of materials in Runs 1, 2, 3, 4, and 5 were 7.4, 7.4, 7.1, 78, and 200 gm. respectively.

Results are shown in Table I. The curves in Fig. 1 are calculated from

TABLE I

REMOVAL OF FLUORINE BY ALUMINIUM OXIDE

*Volume of water treated, litres	F^- content of effluent, p.p.m.				
	Run 1	Run 2	Run 3	Run 4	Run 5
0.5	0.45	0.1	0.1	0.3	0.7
1.0	0.35	0.1	0.15		0.1
1.5	3.0	0.1	0.1	0.2	0.2
2.0		0.3	0.1	0.3	0.3
2.5	7.0	1.3	1.0	0.5	0.4
3.0	8.0	2.0	2.0		1.6
3.5	8.0		3.7	0.2	1.4
4.0			5.0	0.1	1.2
4.5			7.5	0.6	1.5
5.0				0.5	3.0
6.0				0.7	4.5
7.0				0.7	5.0
8.0				1.1	5.5
10.0				3.0	
11.0				4.5	
12.0				7.5	

* *For Runs 4 and 5 multiply volumes by 10.*

Table I in such a manner that abscissae represent litres of water treated by 100 gm. of material and the ordinates represent the fluorine content of the effluent. Litres per 100 gm. of material is roughly the same as gallons per pound.

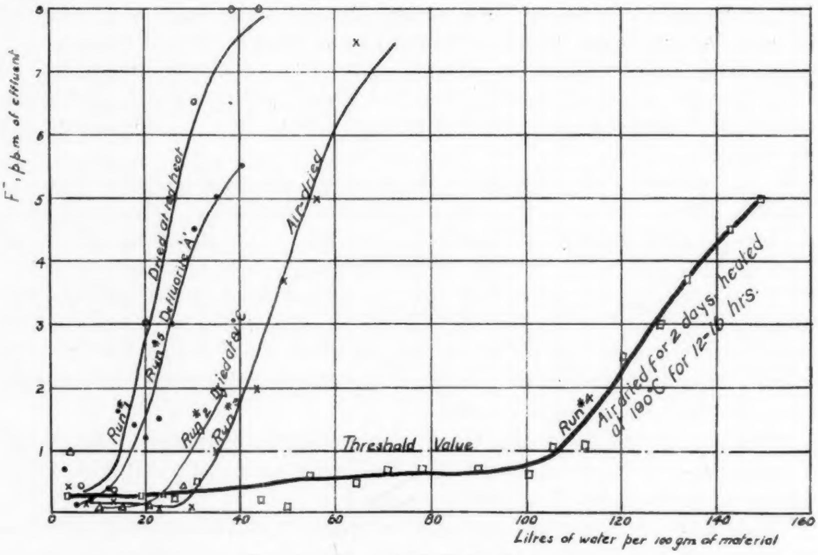

FIG. 1. *Removal of F^- by alumina.*

From Fig. 1 it can be seen that of the five forms of alumina only that used in Run 4 removed sufficient fluorine to justify its use as a fluorine remover. It will be noted that 100 gm. of this particular alumina is capable of lowering the fluorine content of more than 100 litres from 8 p.p.m. to less than 1 p.p.m.

Most of the tests on fluorine removing materials were conducted using the second technique in which a quantity of the material was added to a measured quantity of high fluoride water, stirred thoroughly, and allowed to stand overnight. The treated water was then drawn off and analyzed. A fresh portion of the water was added and treated in the same manner. The procedure was repeated until the fluorine removing power was lost. This technique was favoured because it is more easily followed by the householder in the country and in the small village.

As in the previous series of experiments, good results were obtained from hydrated alumina treated in different ways, from a mixture of co-precipitated alumina and calcium carbonate, and from aluminium phosphate from several sources. For comparison purposes runs were made using magnesium oxide and the commercial preparations of the National Aluminate Corporation, activated alumina here designated as Defluorite *A*, and tricalcium phosphate designated as Defluorite *B*. In addition, experiments have been carried out on tricalcium phosphate from other sources.

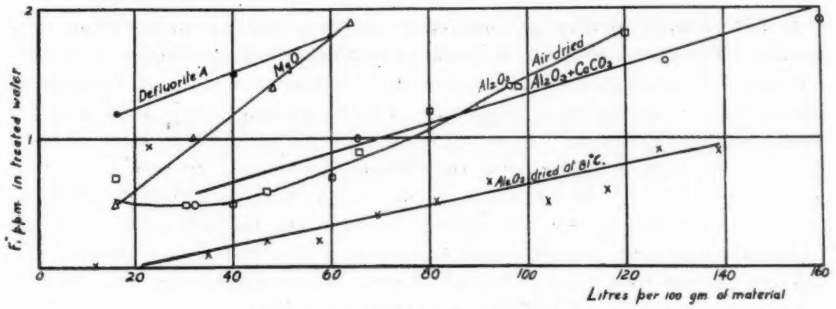

FIG. 2. *Removal of F^- by oxides.*

Table II contains the data obtained and these have been expressed graphically in Figs. 2 and 3. Fig. 2 contains results on oxides, Fig. 3 results on phosphates. In all instances the fluorine content of the water used was 4 p.p.m. except where alumina dried at 81° C. was used, in which case the fluorine content was 3.8 p.p.m. The weight of material used each time was 5 gm. except with alumina dried at 81° C., when 13 gm. was used.

TABLE II

REMOVAL OF FLUORINE BY OXIDES AND PHOSPHATES

F^- content of treated water in p.p.m.

Volume of water, litres	Al_2O_3			$Ca_3(PO_4)_2$		MgO	$AlPO_4$			Al_2O_3 + $CaCO_3$
	Defluorite *A*	Dried at		Defluorite *B*	Baker		Fresh	Baker	Schuchardt	
		Room temp.	81° C.*							
0.8	1.2	0.7		0.0	2.2	0.5	0.2	1.0		
1.5			0							
1.6		0.5		0.6	1.0	1.0	0.2	1.2	0.9	0.5
2.0	1.5	0.5								
2.4		0.6			1.8	1.4	0.6	1.3		
3.0	1.8	0.7	1.0							
3.2		0.9		0.8	2.0	1.9	0.6	2.0	1.8	1.0
4.0		1.2		1.4	1.8		0.8			
4.5			0.1							
4.8		1.4		1.4			1.3		2.6	1.4
5.6				1.3			1.6			
6.0		1.8	0.2							
6.4				1.4			1.3			1.6
7.2				1.8			1.2			
7.5			0.2							
8.0				2.0			1.2			1.9
9.0			0.4							
9.6							1.4			
10.5			0.5							
11.2							2.0			
12.0			0.65							
13.5			0.5							
15.0			0.6							
16.5			0.9							
18.0			0.9							

* *Used water containing 3.8 p.p.m. F^-.*

It will be noticed that in some cases the first result is higher than later results. The materials seem to require a soaking before they begin to behave normally. From Fig. 2 it can be seen that Defluorite *A* is not so effective as some of the other forms of alumina. The magnesium oxide used was not nearly so effective as the variety used by Elvove (6). The alumina dried at 81° C. is the same as that used in the filtration technique, the results of which are shown in Fig. 1. It is much more effective in these experiments probably because of the fact that it was quite gelatinous and did not allow the water to run through very readily in the first series. The mixture of alumina and calcium carbonate is not as effective as some of the other forms. In it, only the alumina is active, as the calcium carbonate has been shown to be incapable of removing fluorides. This mixture was used to obtain a more granular product.

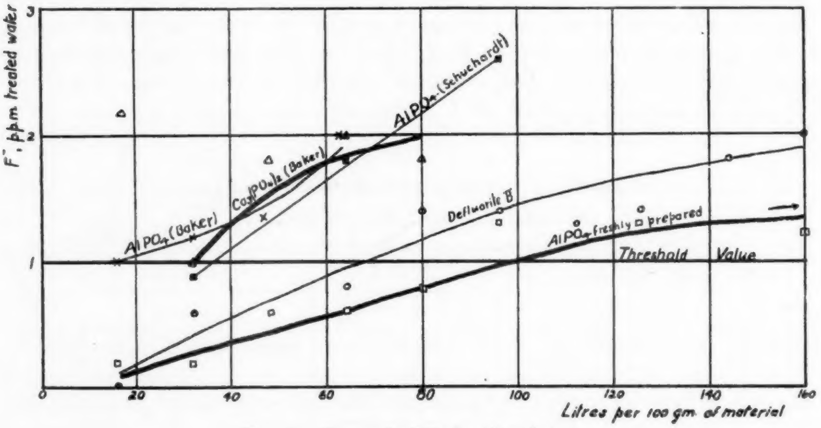

FIG. 3. *Removal of F^- by phosphates.*

From the results in Fig. 3 it can be seen that the effectiveness of freshly prepared aluminium phosphate is of the same order as that of Defluorite *B* and much greater than that of the samples manufactured by Baker and Schuchardt. The capacity of Defluorite *B* is lower with this technique than when the filtration method is used. The aluminium phosphate was prepared by treating aluminium nitrate and monosodium phosphate with ammonium hydroxide, filtering, and then drying in the air. It contained about 45% water. The effectiveness increased somewhat on standing.

From the values given here it can be seen that 1 lb. of alumina dried at 81° C. can lower the fluorine content of 140 gal. of water containing 4 p.p.m. to less than 1 p.p.m., while 1 lb. of the prepared aluminium phosphate can do the same for about 100 gallons. Air dried alumina has also been found to be effective.

It is presumed that if these three preparations remove fluorine from North Saskatchewan River water to which fluorides have been added then they should also be effective with high fluorine natural waters. It is hoped that these materials will be tried out in the near future in areas where mottled teeth is prevalent.

It has been possible, however, to place in service at three points in the province a defluorite unit (1), containing tricalcium phosphate (Defluorite *B*), all of which was kindly furnished by the National Aluminate Corporation. This unit consists of a closed vessel with an inlet tube at the top and an outlet tube at the bottom and contains 10 lb. of 20 to 40 mesh tricalcium phosphate. The material when exhausted may be removed and revivified by sodium hydroxide solution followed by hydrochloric acid or carbon dioxide gas. The unit may be operated under pressure or by gravity. When it is operated under pressure the rate of flow of the water should be no greater than 3 gal. per min. When it is operated by gravity the amount of water treated is about 0.5 gal. per min.

The unit containing fresh material was operated quite satisfactorily at Gwynne, Olds, and Granum. The composition of these waters is given in Table III.

TABLE III

COMPOSITION OF WATER USED IN FLUORINE REMOVAL EXPERIMENTS, IN P.P.M.

——	Gwynne	Olds	Granum
Total solids at 105° C.	964	880	1128
Loss in ignition	212	180	136
$SO_4^{=}$	11	15	342
Cl^-	70	8.6	65
Bicarbonate alkalinity	485	660	325
Carbonate alkalinity	138	6	56
Total hardness (soap method)	0	18	22
$PO_4^{\equiv}$ as P_2O_5	4		0.5
F^-	1.1	3.3	4.4
pH	9.3	8.3	8.55

These three waters are characteristic of many of the high fluorine Alberta waters. In general, it has been found that the pH of such waters is greater than 8.3. Many of them contain considerable quantities of sodium bicarbonate as do the three whose values are shown here.

When defluorite units were operated at these points, a sample of the original water and treated samples taken during the progress of the test were collected and analyzed for fluorine content. In most of these cases it was necessary to conduct the analyses in such a way that phosphates would not interfere, as they were present in the treated water, coming partly from the calcium phosphate.

Table IV contains the results of these experiments.

TABLE IV

REMOVAL OF FLUORIDES BY DEFLUORITE UNIT

—	F^- determined, p.p.m.		
	Gwynne	Olds	Granum
Rate of flow	3 qt. per min.	Fairly rapid	Slow and intermittent
Untreated water	1.1	3.3	4.4
At 50 gal. point			0.1
100 "		0	0.2
200 "	0	0.2	0.2
250 "		0.2	0
300 "		0.4	0.2
350 "		0.5	0.3
400 "	0	0.6	0.2
450 "		0.6	0.2
600 "	0.4	1.0	
800 "	0.4		
1000 "	0.6		
1200 "	0.7		
1400 "	0.6		
1500 "	0.8		

The operation of the units may be looked upon as satisfactory. The materials used at Gwynne and Olds had practically reached the state of exhaustion and had to be regenerated. The unit operated at Granum was very satisfactory and undoubtedly could have been used for treating considerably more of this water.

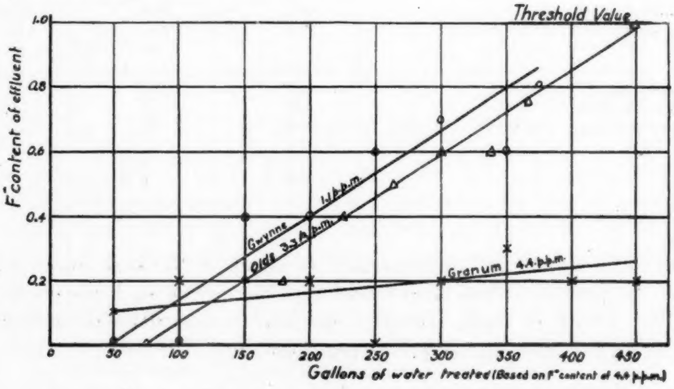

FIG. 4. *Removal of F^- by defluorite unit* [$Ca_3(PO_4)_2$].

The behaviour of these units is expressed graphically in Fig. 4. In this figure the graphs are based on equal amounts of fluorine which passed through the unit. The comparison has been made by taking into account the number of gallons of Gwynne and Olds waters equivalent in fluorine content to 1 gal. of Granum water. For example, 400 gal. of Gwynne water shown in Table IV appears in Fig. 4 as 100 gal. and 400 gal. of Olds water appears as 300 gal. This figure shows that the performance of the unit at Granum was much better than at the other two points. The longer time of contact in the unit may account for the better results.

Summary

It seems clear that the problem of preventing mottled teeth has reached a point where adequate preventives are now available. In addition to those proposed by others, different forms of hydrated alumina as well as aluminium phosphate are added to the list. It is necessary, however, to make careful surveys of endemic regions in order to advise the residents regarding the best methods of prevention. Such surveys have been made in some regions but owing to lack of funds have not yet been made in the province of Alberta. Since persons from other provinces have been observed suffering from mottled enamel it may be a much wider national problem than many suspect.

Acknowledgment

The authors would like to thank the National Aluminate Corporation for the gift of two complete defluorite units.

NOTE.—The methods used to determine fluorine in the work described above will form the subject matter of a subsequent paper.

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DETERMINATION OF THE SPECIFIC SURFACE OF FIBROUS MATERIALS[1]

By E. J. Wiggins[2], W. B. Campbell[3], and O. Maass[4]

Abstract

The Kozeny equation relating specific surface and permeability of a bed of unconsolidated particles has been shown to be applicable to fibrous materials such as glass, wool, and celanese. The diameters of the constituent fibre may be as small as 0.0006 cm., while their ratio of length to diameter may be indefinitely large. Uniformity of size or of packing is not essential.

Introduction

Although fibre surface area is of the greatest importance in most applications of fibrous materials, as yet no satisfactory method is available for its determination. Direct microscopic methods, in addition to tediousness, have obvious objections. The physical form of the fibre is usually so complex as to preclude accurate measurement of the area of any individual, and likewise in most cases the individual fibres in a sample differ considerably in dimensions; this necessitates the measurement of a large number of specimens in order to obtain a fair approximation to the average specific surface. The method to be presented, involving determination of permeability to liquids of a bed of the material, has been investigated with a view to solving this problem.

The basic principles result from an equation relating specific surface and permeability deduced by J. Kozeny (4) from purely theoretical considerations. Carman (2) has stated the equation in a serviceable form and developed experimental procedures for its application. This paper and a preceding one (1) by the same author provide an excellent review of the theoretical considerations of the problem.

The permeability, k, is defined as the linear rate of flow of liquid through the bed under unit hydraulic gradient.

$$K = \frac{Q}{A} \cdot \frac{L}{h} \tag{1}$$

Where Q = quantity of liquid flowing through bed in unit time;
A = area of cross section of bed;
L = depth of bed;
h = head of liquid in consistent units.

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Contribution from the Department of Physical Chemistry, McGill University, Montreal, Canada. This investigation was carried out in co-operation with the Forest Products Laboratories of Canada, Montreal, and formed part of the research program of that institution.

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Equation (1) is merely a statement of D'Arcy's law, namely, that the linear rate of flow through a porous medium is proportional to the pressure gradient across the bed.

The relation between permeability and specific surface, termed the "Kozeny equation", is given by Carman as follows:—

$$S_0 = 14\sqrt{\frac{1}{K\nu}\,\frac{\epsilon^3}{(1-\epsilon)^2}} \tag{2}$$

Where S_0 = "specific surface" = surface area per unit volume of actual material comprising the bed;

K = permeability as before;

ν = kinematic viscosity = $\dfrac{\text{absolute viscosity}}{\text{density of liquid}}$;

ϵ = "porosity" = free volume in bed expressed as a fraction of the total volume.

Note $S_0 = \dfrac{S}{1-\epsilon}$, where S = surface area per unit volume of bed.

Equation (2) is valid only for the condition of viscous or streamline flow, the criterion being that

$$\frac{Q}{A\nu S} < 2\ . \tag{3}$$

However, this condition is amply fulfilled for all cases encountered in the present investigation. The great value of the Kozeny equation, if substantiated, as a means of determining fibre area lies in its validity for any shape of particle, and in the ability of the term $\dfrac{\epsilon^3}{(1-\epsilon)^2}$ to correct for various degrees of porosity of the bed. An extensive investigation of the effects of variation of particle size, porosity of bed and liquid viscosity was conducted by Carman, the experimental results indicating that the equation expresses the variables in correct relation. Variation of particle shape and the mixing of various sizes provided further evidence in its favour. However, the most extreme departure from spherical form was represented by crimped wire of 0.03 cm. diameter and straight length 0.57 cm., so that further experimental study was necessary before applying the equation with assurance to particles of the type found in ordinary fibrous materials.

Experimental Procedure

The apparatus employed for the permeability determination is illustrated in Fig. 1, and is a modified form of that used by Carman.

The bed of particles is packed in the tube *A* and rests on a 20-mesh copper gauze *B*, which is in turn supported by a spiral of thin sheet copper *C*. The tube *A* is of as uniform cross section as possible, and is calibrated by filling both limbs with water, closing *D*, and weighing the water run out for a series of levels in *A*. A fixed head of liquid is maintained on the bed by a common

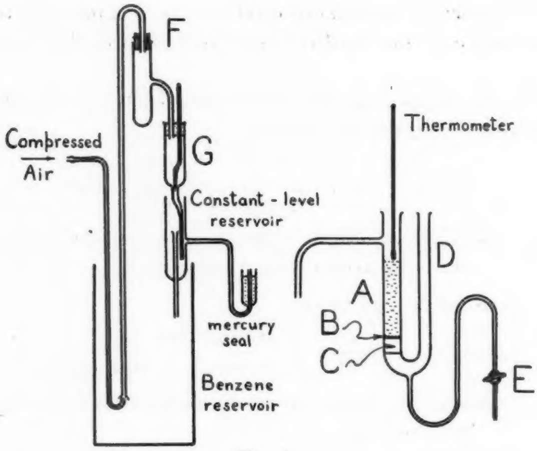

FIG. 1.

overflow type, constant-level device. Water and benzene were used in the investigations, the latter requiring a mercury seal for the connection between permeability tube and constant level reservoir, as shown at the left of Fig. 1. The overflowing benzene is recirculated by means of an air-lift, the intervening reservoirs *F* and *G* serving to smooth the flow and allow entrained air to escape.

The rate of flow of liquid is controlled by means of stopcock *E*, and the corresponding pressure drop across the bed obtained from the difference of liquid levels in tubes *A* and *D*. A minor scale, graduated in 1 mm. divisions, is used to read the liquid level in *D*, the level in *A* being transferred to the same scale by means of a sliding index; this scale is also used to measure the depth of the bed. The rate of flow is obtained by weighing the liquid passing through the bed during a definite time interval. Density and viscosity data for the liquids used were obtained from standard tables. The apparatus was not thermostatically controlled, but the constancy of the ambient temperature was such as to enable reading the temperature of the liquid to 0.1° C., well within the required limits.

The bed is prepared by filling the permeability tube with the liquid to be circulated, and introducing a weighed quantity of the solid material with constant stirring. Considerable difficulty was encountered in obtaining a bed free from entrained air, especially with the more fibrous particles; in such cases the side arms were closed and suction applied to the bed.

The densities of the various materials used were determined by displacement of benzene or water in a pycnometer flask. The volumes of solids were calculated from the weight and density of the particles, and the total volume of the bed from its depth and cross section; from the two values the porosity was obtained.

Experimental Results

1. Preliminary measurements were made with beds of screen-classified sand, similar to those used by Carman. Since the actual specific surface of the sand particles was not known, these determinations served mainly to show the constancy of the calculated values with different liquid viscosities and depths of bed. It was also possible to note how closely the variation of specific surface from a fraction of particles of one size to another compared with that calculated from the ratios of average particle diameters.

Typical results are listed in Table I.

TABLE I

Size range	Liquid	Average particle diameter, cm.	$K\nu$	ϵ	S_0, cm.^{2}/cm.^{3}	S_0d
−10 +14 mesh	Water	0.141	1.163×10^{-2}	0.440	67.9	9.58
−20 +28	Water	.0711	4.19×10^{-3}	0.430	116.8	8.30
−28 +35	Water	.0503	2.15×10^{-3}	0.443	160.8	8.10
−28 +35	Benzene	.0503	2.58×10^{-3}	0.456	157.0	7.90
−35 +48	Water	.0356	0.928×10^{-3}	0.436	235	8.38
−48 +65	Water	.0252	0.561×10^{-3}	0.430	293	7.37

If the shapes of the sand grains were identical over the entire size range, the specific surface should vary inversely as the average diameter, and thus the product S_0d, where d = diameter of particle, would be a constant. The departure actually shown in the upper and lower fractions was probably an indication of variation in average shape, microscopic examination indeed showing the larger particles to be nearly spherical and the smaller sizes more complex.

2. To obtain particles in a form resembling that of fibres, heated glass rod was pulled out to an approximate diameter of 0.04 cm. The resulting strands were sorted, cut into 8 mm. lengths, and the diameters of a random sample of 100 measured with a micrometer. A weighed quantity of the glass was then packed into the tube A, and the permeability of the bed to water determined.

Experimental data were as follows—

Permeability $K\nu$ (average of 10 runs) $= 4.78 \times 10^{-2}$

Porosity $\epsilon = 0.685$

Calculated $S_0 = 115$ cm.^{2}/cm.^{3}

Average diameter of strands = 0.0407 cm. (extreme variation = 0.0275 to 0.0525 cm.)

Ratio of length to diameter $L/d = 20$

Actual $S_0 = 101$ cm.^{2}/cm.^{3} Ratio of calculated value to measured value = 1.14.

3. The permeability to water of a bed of No. 38 enameled copper wire (5 mm. lengths) was determined as before.

Permeability $K\nu$ (average of 22 runs) $= 2.14 \times 10^{-2}$
Porosity $\epsilon = 0.830$
Calculated $S_0 = 433$ cm.^{2}/cm.^{3}
Diameter of wire $= 0.01017$ cm. L/d ratio $= 50$
Actual $S_0 = 394$ cm.^{2}/cm.^{3} Ratio of calculated value to measured value $= 1.10$

4. To obtain greater departure from sphericity and more closely simulate the physical form of actual fibres, beds of ordinary glass wool were investigated. Permeability determination were made with both water and benzene on beds consisting of glass wool in 1.5 and 6 mm. lengths. Since rod-like particles of this kind would show a tendency to orientation during the packing process, beds were prepared both by random packing and by allowing the fibres to settle after suspension in the liquid with mechanical agitation. Any appreciable effect should then be evident from a comparison of the two results.

Paraffin-block sections were prepared from a sample of the glass wool, and the cross sectional area and perimeter calculated from microscopic measurements. The cross section proving to be of hour glass form, the outline was plotted on graph paper for this purpose.

TABLE II

Fibre length, mm.	L/d ratio	Liquid	$K\nu$	ϵ	S_0, cm.^{2}/cm.^{3}	Remarks	$\frac{\text{Calculated value}}{\text{Measured value}}$
6	200	Benzene	4.70×10^{-3}	0.910	1965	Random packing	0.894
6	200	Water	2.95×10^{-3}	0.895	2090	Random packing	0.950
6	200	Water	1.106×10^{-3}	0.846	2130	Natural settling	0.968
1½	50	Water	1.294×10^{-3}	0.857	2165	Natural settling	0.985

Specific surface calculated from microscopic examination—2200 cm.^{2}/cm.^{3} (for a uniform fibre S_0 = ratio of perimeter to cross sectional area).

5. To obtain still greater ratios of length to diameter and smaller actual diameters, Corning "fibreglass" No. 008 was employed. Three different types of bed were used, the fibreglass being first cut into 1.5 mm. or 8 to 10 mm. lengths, or merely packed into the tubes without preliminary treatment. Permeability determinations were made with both water and benzene. The fibre cross sectional area and perimeter were determined from microscopic measurements as with the larger glass wool.

6. To test the validity of the equation when a considerable size variation existed in the bed, approximately equal quantities of fibreglass and glass wool, each in 1.5 mm. lengths, were thoroughly mixed.

TABLE III

Fibre length, mm.	Approx. L/d ratio	Liquid	$K\nu$	ϵ	S_0	$\frac{\text{Calculated value}}{\text{Measured value}}$
1½	200	Benzene	2.32×10^{-4}	0.885	6660	0.95
8–10	1000	Benzene	4.08×10^{-4}	0.919	7540	1.1
Continuous		Benzene	5.30×10^{-4}	0.928	7560	1.1
Continuous		Water	4.97×10^{-4}	0.930	8070	1.2

Actual specific surface $= 7 \times 10^3$ *cm.²/cm.³*

Results of permeability measurements with water on the mixture were as follows:—

Permeability $K\nu$ (average of 14 runs) $= 7.62 \times 10^{-4}$

Porosity $\epsilon = 0.909$

Calculated $S_0 = 4830$ cm.^{2}/cm.^{3}

Actual S_0 (calculated from relative quantities and specific surfaces of fibreglass and glass wool) $= 4710$ cm.^{2}/cm.^{3} Ratio of calculated value to measured value $= 1.03$

7. Celanese yarn was cut into 5 mm. lengths, the filaments were unravelled by rubbing, and the resulting material was formed into a bed; the permeability to benzene was determined in the usual manner. The cross sectional areas and perimeters of the filaments were estimated from microscopic measurements with the aid of drawings of the outline. However, owing to the complex form, the calculated surface area could not be relied on to be of greater accuracy than ±20%.

The apparent density of the fibres was obtained by simple displacement of benzene in a pycnometer flask. This determination was carried out with the celanese in equilibrium with room humidity, as were also the permeability and area measurements. While the atmospheric humidity was undoubtedly subject to some fluctuation, it was assumed that the variation of moisture content from this cause would not introduce serious error. It was further assumed in determining the superficial density by displacement of benzene that none of this liquid would be adsorbed by the celanese.

TABLE IV

Run	Average $K\nu$	ϵ	S_0, cm.^{2}/cm.^{3}	$\frac{\text{Calculated value}}{\text{Measured value}}$
1	1.574×10^{-3}	0.898	2950	1.0
2	1.749×10^{-3}	0.904	2990	1.0

Actual $S_0 = 3.0 \pm 0.5 \times 10^3$ *cm.²/cm.³*

Discussion

In the preceding section, substantial agreement is shown between the values of specific surface as calculated from permeability determinations and from the geometry of the particle. In the size ranges of most interest for future practical application, namely fibreglass and celanese, the agreement is within the accuracy of the determination of actual surface area.

The presence of more than one size of particle, at least when in approximately equal quantities, does not affect the applicability of the method. Furthermore, non-uniform packing of the bed does not seem to be a troublesome factor. This is striking in view of the wide variation of the $\frac{\epsilon^3}{(1-\epsilon)^2}$ term in the Kozeny equation for relatively small changes in ϵ, from which it might be thought that the effect of the more closely packed portions of the bed on the permeability would not be counterbalanced by the looser sections.

The nature of the particle surface in contact with the liquid is found to exert no influence on the permeability for such widely different materials as copper, glass, and celanese; also for both polar and non-polar liquids as water and benzene.

The results of this investigation indicate that the Kozeny equation may be confidently applied to the determination of the specific surface of materials of the type mentioned, with indefinitely large L/d ratios, and with fibre diameters as small as 0.0006 cm. Size mixtures are admissible at least within the range 5 : 1 and where the relative quantities are not widely different. In a recent paper (3) Carman has shown that satisfactory results are obtainable from a mixture of very wide size variation, so that it appears probable that the Kozeny equation would be equally applicable to fibre mixtures.

Further investigation is necessary in order to extend the study to non-rigid materials of more complex structure, such as natural cellulose fibres. A further complication is here introduced in that swelling effects are of the greatest importance. This work is being undertaken at the present time with the ultimate object of determining the surface area of wood pulp fibres, and possibly correlating with this the results from the commercial "freeness tester".

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A MODIFIED PROCEDURE FOR THE DETERMINATION OF CAROTENE IN SILAGE[1]

By A. C. Neish[2], W. D. McFarlane[3], and W. A. Brechin[4]

Abstract

The pigments in silage which have been formed from xanthophyll by acids and which interfere in the determination of carotene have been separated by chromatographic methods and found to exhibit solubilities intermediate between those of carotene and xanthophyll. A method based on the partition coefficient of carotene and the interfering pigments in the system hexane-benzyl alcohol was found to give reliable estimates of carotene.

Introduction

It has frequently been observed that silage made from freshly cut green herbage when analyzed by the conventional methods may appear to contain more carotene than was found in the original plant material (3, 4, 6–9, 11). Quackenbush, Steenbock, and Peterson (8) have recently made a thorough study, by chromatographic analysis, of the pigments present in acid treated alfalfa silage. Five new pigments were identified which were not present in the untreated forage and three of these were shown to have been formed from xanthophyll by the action of acid, and to closely resemble carotene in their solubilities.

The writers have analyzed samples of silage for their carotene and xanthophyll content with the results shown in Table I. The determinations were made by the method of Guilbert (1); a 10 gm. sample of the fresh silage or a 1 gm. sample of the dried material was used and the final colour intensity measurements were made with an Evelyn photoelectric colorimeter (440 filter). The clover silage was prepared from freshly cut red and alsike clover—alfalfa aftermath which had flowered and which was found to contain 0.026 mg. of carotene per gm. of dry matter. The fall rye, cut when about fifteen inches high, analyzed 0.238 mg. of carotene per gm. dry matter. Compared with these values for the original herbage, the results in Table I indicate an increase in carotene due to ensiling the clover and a decrease in the case of fall rye. The carotene analyses of the herbage were carried out with vacuum dried samples, and the results may therefore be low. Under the conditions employed, carotene was definitely destroyed during the ensiling of fall rye.

Drying the silage *in vacuo* for 12 hr. at 90° C. results in a very marked increase in the apparent carotene content and a corresponding decrease in xanthophyll. This observation was made use of in the experiments to be described below. A simple procedure is described for separating carotene from the interfering pigments formed from xanthophyll, thus making possible the determination of carotene in silage.

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TABLE I

CAROTENE AND XANTHOPHYLL DETERMINATIONS ON SILAGE, SHOWING THE EFFECT OF DRYING THE SAMPLE FOR 12 HR. *in vacuo* AT 90° C.

(Results expressed in milligrams per gram dry matter)

Sample	pH	Fresh			Dried		
		Carotene	Xanthophyll	*c/x* Ratio	Carotene	Xanthophyll	*c/x* Ratio
Clover silage:							
(1) No treatment	5.18	0.054	0.388	0.139	0.201	0.240	0.840
(2) Phosphoric acid*	4.37	.040	.366	.110	.255	.180	1.419
(3) Molasses**	4.40	.042	.374	.112	.225	.167	1.349
Fall rye silage:							
(4) Phosphoric acid	4.27	.163	.668	.244	.471	.300	1.570
(5) Molasses	4.40	.137	.615	.233	.375	.260	1.445

**9 litres of 32.5% phosphoric acid was added to 1000 lb. of herbage.*

*** 40 lb. of molasses dissolved in 20 lb. of water was added to 1000 lb. of herbage.*

Experimental

Large samples of fresh and dried fall rye silage, representing equal amounts of dry matter, were refluxed with a 20% solution of potassium hydroxide in ethyl alcohol for one hour, and the carotenoid pigments extracted with petrol ether. The petrol ether extract was washed with water and the xanthophyll next extracted by shaking with 92% (by volume) ethyl alcohol; three washings gave a distinct separation. The petrol ether fraction was concentrated *in vacuo* and put through a column of magnesia and siliceous earth according to the method of Strain (10). The carotene was washed through the column with petrol ether, whereas the other pigments were tenaciously adsorbed in two bands at the top of the column; the upper was greenish-yellow and the lower orange-yellow. The latter could be separated into two orange-yellow adsorption bands by washing slowly with a mixture of ethanol (1% by volume) and petrol ether. These findings are in agreement with the observations of Quackenbush *et al.* (8). A much larger quantity of these pigments was contained in the chromatogram from the heat treated silage. It would appear also that the presence of these "*X*" pigments in plant material is not confined to silage since they were found to be present, in considerable amounts, in a chromatogram of the carotenoid pigments extracted from a sample of fall-rye herbage which had been dried *in vacuo*.

After the carotene had been removed the remaining pigments were eluted with absolute ethanol; these are referred to below as "*X*" pigment. A comparison was made of the properties of a solution of crystalline β-carotene and of the pigment solutions prepared from silage to find some difference on which a method for the determination of carotene in the presence of "*X*" pigment might be based. No significant difference was observed in the colour reaction with antimony trichloride or in the rate of oxidation with benzoyl peroxide.

However, considerable difference was noted in the phase distribution of carotene and "*X*" pigment between petrol ether and the following solvents:—benzyl alcohol, allyl alcohol, furfuryl alcohol, phenyl ethyl alcohol, and dimethyl phthalate. In each case, carotene remains chiefly in the petrol ether whereas the greater part of the "*X*" pigment goes into the other solvent, the latter therefore showing solubilities intermediate between those of carotene and xanthophyll.

Benzyl alcohol was considered the best solvent to use because it gave a good separation and is more readily procurable and less expensive than the other solvents. Petrol ether was replaced by hexane, which has a more definite composition. Because of the incomplete separation of the pigments it was considered advisable to make one phase separation and calculate the amount of carotene present from experimental data on its distribution in a hexane-benzyl alcohol system. Benzyl alcohol dissolves hexane, the miscibility depending on temperature. If 8 ml. of benzyl alcohol and 12 ml. of hexane are mixed at 20° C., each phase at equilibrium has a volume of 10 ml. For convenience therefore these volumes of the two solvents were employed.

METHOD FOR THE DETERMINATION OF CAROTENE, XANTHOPHYLL AND "*X*" PIGMENT IN SILAGE

The silage should be properly sampled as soon as it is taken from the silo and the analyses carried out without delay, because the carotene content diminishes on exposure of the material to the air.

A 10 gm. sample is placed in a 300 ml. Florence flask, 30 ml. of 50% aqueous potassium hydroxide and 60 ml. of 95% ethanol are added, and the mixture is refluxed for one hour on a water bath. The material is transferred to a 250 ml. centrifuge bottle, and the flask rinsed out with water; 50 ml. of ethyl ether (peroxide-free) is added; the contents of the stoppered bottle shaken for two or three minutes, and, after centrifuging, the ether layer is suctioned off into a 300 ml. separatory funnel. Four more extractions with ethyl ether are made using two 50 ml. and two 30 ml. portions. The combined ether extracts are washed five times with water; the ether is distilled off *in vacuo* and 70 ml. of hexane introduced into the flask before the vacuum is released. If the carotene content only is to be determined, hexane may be substituted for ethyl ether; thus the distillation may be avoided. The solution is finally transferred to the separatory funnel using 70 ml. of 92% (by volume) ethanol. The xanthophyll is washed from the hexane solution with 92% (by volume) ethanol, four washings usually giving complete extraction. The washings are combined in a 250 ml. volumetric flask, diluted to volume, and the xanthophyll content is determined by photoelectric colorimetry, the results being expressed in terms of carotene by reference to a graph obtained with solutions of pure carotene.

The hexane solution is transferred to a 100 ml. volumetric flask, diluted to volume, and its carotene and "*X*" pigment content determined as follows:—

A portion (12 ml.) of the hexane solution is placed in a colorimeter tube; the colour intensity is measured with an Evelyn photoelectric colorimeter (440 filter) and the pigment concentration expressed in terms of the carotene reference graph. To the hexane solution is added 8 ml. of benzyl alcohol, the mixture shaken thoroughly, and placed in a water bath at 20° C. until a clear separation of the two phases occurs. The pigment present in the hexane phase is then determined using the colorimeter attachment described by Parker and Griffin (5), which made it unnecessary to remove the hexane phase from the benzyl alcohol phase.

If T represents the total carotenoid in the system and H represents the carotenoid in the hexane phase, then $T - H$ will approximate the "X" pigment content of the system because of the low solubility of the "X" pigment in hexane (see Fig. 1). Knowing the value $(T - H)$ a correction can be made for the amount of "X" pigment in H by reference to Fig. 1, which shows the distribution of "X" pigment in the system. Then H − ("X" present in hexane phase) gives the true carotene content of the hexane phase. The carotene content of the system can now be read from Fig. 1, which shows the distribution of carotene in the system. The true value for "X" pigment is the difference between the total carotenoid and carotene values.

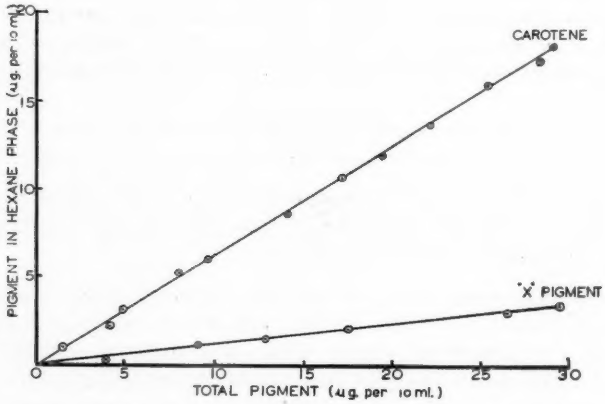

FIG. 1. *Phase distribution of carotene and "X" pigments in the system benzyl-alcohol–hexane.*

Discussion

Analysis of mixtures of carotene and "X" pigment (Table II) showed that fairly accurate values are obtained for the carotene content of the system, provided that the "X" pigment : carotene ratio does not exceed 2, a value which is not likely to be found in silage. The five samples of silage referred to in Table I were analyzed for carotene, xanthophyll, and "X" pigment by this procedure. The vacuum-dried samples were likewise analyzed. Thus an indication was afforded of the accuracy with which carotene could be estimated in the presence of a relatively large amount of "*X*" pigment.

TABLE II

SHOWING THE RECOVERY OF CAROTENE FROM MIXTURES OF CAROTENE AND "*X*" PIGMENT AS CALCULATED FROM THE PHASE DISTRIBUTION

Total carotenoids, µg.	Ratio ("*X*" pigment/ carotene)	Carotene, µg.	
		Present	Found
33.6	0.41	23.8	23.8
22.8	0.63	14.0	13.6
15.8	0.71	9.2	9.4
39.6	0.76	22.5	22.8
29.6	0.95	15.2	15.0
26.4	1.13	12.4	12.0
9.6	2.20	3.0	2.9
19.2	2.20	6.0	6.6
16.2	3.88	3.4	4.6

From the results summarized in Table III it will be seen that in all these samples of silage the amount of "*X*" pigments is relatively small, representing approximately 10% of the carotene content. The validity of the method proposed for determining carotene in the presence of the acid decomposition products of xanthophyll is supported by these results. Drying *in vacuo* resulted in a marked destruction of xanthophyll, whereas carotene was remarkably stable. Only about 35% of the xanthophyll lost by drying was recovered as "*X*" pigment. This may be due in part to the fact that the concentration of all the pigments is expressed in terms of the absorption by carotene of light of wave-lengths approximating 440 mu. The absorption coefficient of "*X*" pigment may be much less than carotene. A sample of silage No. 5 was heated in a sealed glass tube for 24 hr. at 90° C. In this way heating was accomplished without drying. The results of the analysis of this material were practically identical with those of the vacuum-dried sample.

TABLE III

CAROTENE, XANTHOPHYLL, AND "*X*" PIGMENT CONTENT OF SILAGE

(Results expressed in micrograms per gram dry matter)

Sample	Xanthophyll		Carotene		"*X*" pigment	
	Fresh	Dried	Fresh	Dried	Fresh	Dried
1	366	288	23	23	3	38
2	358	238	24	26	3	32
3	367	258	31	33	6	47
4	525	379	57	55	2	34
5	483	392	44	50	5	45

Acknowledgments

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Note added July 24, 1939

Since the completion of this work, Hegsted, Porter, and Peterson (2) have published a method for the determination of the carotene content of silage in which diacetone is employed to remove the pigments other than carotene. Some carotene is lost in the diacetone extract, as it is on extraction with benzyl alcohol, an objection which caused us to discard the use of repeated extractions with this solvent in favour of a single phase separation. They claim however that the loss of carotene in the diacetone extract is offset by the amount of non-carotene pigment which remains in the carotene fraction. We intend to compare this method with our own procedure at the first opportunity.

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